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Meristem Initial Cells in Irradiated Roots of *Vicia faba*

BY

D. DAVIDSON

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With one Figure in the Text

Abstract

Beans (*Vicia faba*) were germinated for 24 hours and irradiated with X-rays. Primary roots were fixed after 9, 11, and 21 days. Aberrant chromosome complements, the result of chromosome changes induced by irradiation, were present. They were used as cell markers to estimate the number of cell types present in the meristem initial cells of the growing root and the number of primordium initial cells from which root regeneration occurred. Up to 9 cell types occur as meristem initials. From the relative frequencies of the different cell types, it is estimated that there are at least 32 actual meristem initial cells in regenerating irradiated primary roots. This result is compatible with observations made on normal roots. The chimaerical nature of the regenerating root apparently does not interfere with the normal organization of the meristem, but it serves to reveal what part of the organization is.

Introduction

THE majority of the cells with chromosome aberrations found in division after X-irradiation of roots undergo one or only a few mitotic divisions. Chromosome aberrations do not continue to appear indefinitely in meristem cells (Sax, 1941; Nichols, 1941; Thoday, 1951) since many of them are cell lethal, and micronuclei, the result of lagging chromosome fragments at anaphase, gradually disappear from irradiated meristems (Gray and Scholes, 1951). A few changed chromosomes may, however, be found in roots several days or weeks after irradiation (Sax, 1941; Nichols, 1941; Brumfield, 1943; Davidson, 1959*a*, *b*). The most readily distinguishable type of changed chromosome is the dicentric; but this is also the most unstable, because of bridge formation at anaphase, and it was found to disappear quickly from root cells (Sax, 1941). Though some chromosome aberrations undoubtedly lead to cell death and are therefore seen only once, other chromosome changes are not lethal. Reciprocal translocations can survive many cell divisions and give rise to multivalent associations at meiosis; minute deficiencies are not all lethal, even when homozygous (Stadler, 1941; McClintock, 1944) and even large deficiencies or the nullisomic condition (McClintock, 1929) may not prevent a cell from dividing. Deficiencies were not found in roots of *Allium* several days after irradiation, or in primary roots of *Vicia*, 3 weeks after irradiation (Brumfield, 1943); but they have been described from the lateral roots of *Vicia* that grew from primary roots 3–4 weeks after irradiation (Davidson, 1959*a*).

[1] Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

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Translocations, inversions, and deficiencies are not all cell lethal and can form stable new chromosomes. Cells with such chromosomes are marked and can be distinguished from cells with other chromosome complements. Such cell markers have been used to follow cell behaviour:

1. They were used to estimate the number of root initial cells in primary roots (Brumfield, 1943) and in lateral roots of *Vicia* (Davidson, 1959*a*).
2. They were used to estimate the minimum number of mitotic divisions of certain cells in the period between irradiation and fixation (Davidson, 1959*b*).

The estimates of the number of initial cells (there appear to be six or seven for lateral roots and only three for primary roots of *Vicia*) were obtained with different experimental procedures; the lateral roots were prepared as squashes and were derived from roots that had received 600 r, while the primary roots were sectioned and had received only 300 r. In re-examining the survival of cells with chromosome markers in primary roots, an attempt has been made to resolve the differences between lateral and primary roots, and also to reconcile the cytological evidence, of a small number of initial cells, with the recent anatomical evidence of a much larger number of initial cells (Clowes, 1956; Jensen and Kavaljian, 1958).

The experiments to be described were undertaken in order to determine the number of meristem initial cells from which a primary root of *Vicia* was regenerated after irradiation.

Material and Methods

Beans (Sutton's Exhibition Long Pod) were irradiated after 24 hours' soaking. The X-ray exposure dose of 600 r was delivered, in 3 minutes at 60 cm. from a G.E. Maxitron, through a 3-mm. aluminium filter at 250 kVp and 30 mA. (H.V.L. = 0·54 mm. of Cu). The beans were grown over distilled water. The water was changed every day and was aerated, with air, continuously. Primary roots were fixed in 2BD 9, 11, and 21 days after irradiation. Immediately before fixation they were pretreated with 0·05 per cent. colchicine for 3 hrs., then bleached, hydrolyzed in 1·0 N.HCl, and prepared as Feulgen squashes.

The capacity of the culture tank was 12 litres. With roots grown for 21 days, 1 litre of standard Hoagland's solution was added to the tank every day after the 10th day to supply necessary minerals.

Root length was measured, from a point at the joint of the root and shoot, in roots fixed 21 days after irradiation. The data gave an indication of the ability of the irradiated roots to form new growth.

Of 18 roots fixed at 9 and 11 days, 16 had cells in division, 8 for each day. At 21 days 33 beans were fixed; 10 roots were taken at random, and all had cells undergoing mitosis. Chromosome complements were analysed at metaphase. *Vicia* has 10 short chromosomes with subterminal centromeres and 2 long chromosomes with median centromeres, i.e. S and M chromosomes.

Stable Changed Chromosomes

Cells with stable changed chromosomes were found 9, 11, and 21 days after irradiation. The new chromosomes were the result of inversion, reciprocal translocation, or deletion; new complements were also found in which there were additional chromosomes (Fig. 1). Most marked cells contained only a single change, but cells with a double change were found (Davidson, 1959*b*, Fig. 1). It is a basic assumption in this study that all cells in a root with a particular change are descended from one cell. If this assumption is rejected,

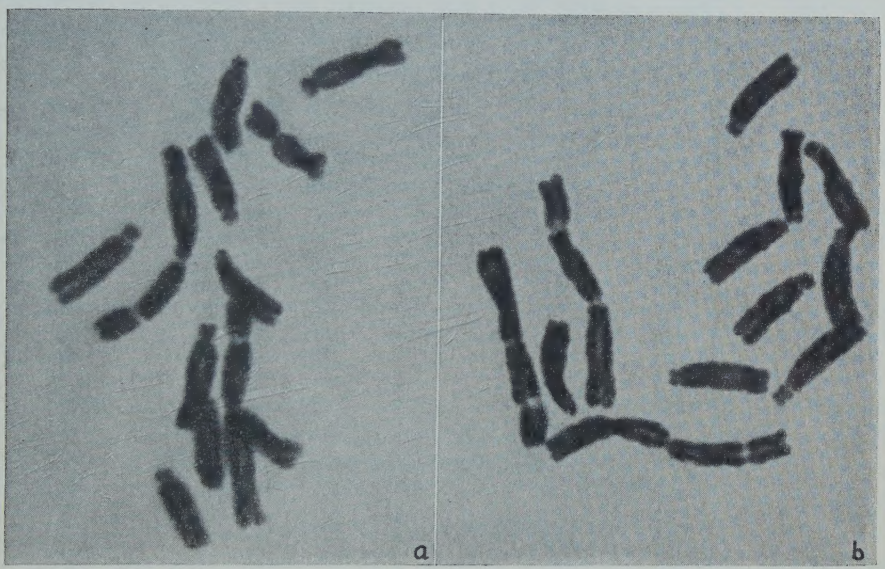

Fig. 1. New chromosome complements in cells of primary roots of *Vicia* fixed 21 days after 600 r. (*a*) An inversion in an S chromosome has converted it to one with a submedian centromere. (*b*) The complement contains 9 S and 3 M chromosomes. ×1,800.

it would seem that each root exhibits a unique case of localized breakage and reunion after X-irradiation; this seems unlikely since it can hardly explain the presence of a number of cells, each with the same double change or additional chromosomes.

The results from roots from all three fixation times (Tables 1 and 2) show that cells with new complements do perpetuate themselves. In the earliest fixation, one root was found with twelve detectable types of cells active in its meristem (Table 1, root 15: 11 types of changed cells and 1 normal cell). The number of initial cells with normal complements that were actively dividing cannot, of course, be estimated by comparing chromosome complements. The number of initial cells that can be postulated from a study of the presence of new complements yields no information on the stability of the cytologically chimaerical root, nor, since cells were examined in squash preparations, can we know anything of their original arrangement in the root. It could, therefore, be argued, that roots fixed 9 and 11 days after irradiation, that is, soon

after the roots began to grow again, were unstable chimaeras and that the cells with chromosome markers scored were the last survivors present in the meristem and were not descended from similarly labelled initial cells. On this basis it would be fortuitous that the same aberration was present in several cells in a root, or that the same aberration occurred in both parts of a split root (Table 1, root 18, Davidson, 1959*b*). The argument that the roots are

TABLE 1

The Frequency of Cells with Normal or Aberrant Complements in Apical Meristems of Primary Roots of Vicia *that Received 600 r after 24 hrs. Germination. Roots were Fixed after 9 or 11 Days. Roots with the same Number in Tables 1 and 2 Represent Roots from Different Beans*

Root*	Cells		No. of changed complements	Per cent. changed cells	Total metaphases analysed
	Normal	Changed			
	9 Days after X-irradiation				
15	158	46	11	22·33	204
12	171	47	10	21·55	218
3	125	13	6	10·42	138
8	42	11	7	20·7	53
4	42	—	—	—	42
14	25	2	1	10·7	27
6–1†	41	3	1	6·81	44
6–2†	71	10	4	12·34	81
18–1†	131	55	5	29·56	186
18–2†	75	27	1	26·47	102
	11 Days after X-irradiation				
11–1†	76	9	4	10·5	85
11–2†	74	7	2	8·64	81
1	106	13	4	10·92	119
5	97	21	5	17·79	118
10–1†	72	6	3	7·69	78
10–2†	62	17	3	21·51	79
9	125	38	8	23·31	163
2	139	63	10	31·18	202
13	70	11	5	13·58	81
11	68	14	2	17·07	82
Total	1,770	413		Av. 18·91	2,183

* Roots 7 and 16 were without divisions. † Roots with a split apex.

unstable chimaeras cannot be applied to the roots fixed 21 days after irradiation: the fact that the percentage number of changed cells per root is not smaller at 21 than at 9 or 11 days, that the roots are long, and that one root contained a majority of dividing cells with changed complements do not indicate that the roots are highly unstable. If the roots are reverting to their original condition, with all cells having identical complements, they must be doing so very slowly, i.e. in more than 21 days; and the period in which it might be considered that the cells with the same aberrant chromosomes are decreasing in frequency in the meristem, must follow a period, immediately after irradiation, in which such cells obviously increase in frequency in the meristem. The roots fixed 21 days after X-irradiation appear to be fairly

stable and the presence of aberrant chromosomes in the cells of the meristem of the regenerated root is evidence that cells with identical complements occur as root initials. ('Regenerated' is used to denote a new outgrowth from a root whose growth had ceased after irradiation; not that a root had been physically decapitated.)

TABLE 2

As 1; Roots Fixed after 21 Days

Root	Root length (cm.)	Cells		No. of chromosome changes	Per cent. changed cells	Total metaphases analysed
		Normal	Changed			
1	10·5	108	46	6	23·3	154
14	17·5	84	26	7	23·6	110
19	9·0	245	72	7	22·7	317
11	9·6	63	20	2	24·1	83
4	9·0	212	111	8	34·4	323
23*–1	10·0‡	84	34	1	29·9	118
23–2	7·5‡	79	—	—	—	79
10	12·4	71	13	3	15·5	84
20†–1	1·5‡	9	48	1	84·2	57
20–2	2·1‡	47	14	1	22·9	61
20–3	1·4‡	35	—	—	—	35
20–4	1·9‡	30	—	—	—	30
25	9·1	75	62	8	22·1	137
31	16·7	108	17	3	13·6	125
Total		1,250	463		Av. 27·03	1,712

* The root was 3·4 cm. long before it split.
† The root was 1·5 cm. long before it split.
‡ Measured from the point at which the root split.

SPONTANEOUS ABERRATIONS

Most roots will reveal evidence of spontaneous chromosome aberrations: breakage may be found in cells undergoing mitosis, or micronuclei in resting cells. The frequency of spontaneous aberrations in roots examined in this study was low. There were 0·05 per cent. chromosomes with breaks in 9- and 11-day fixations, 0·027 per cent. in 21-day fixations.

Spontaneous aberrations could lead to the presence in the meristem of cells with chromosome changes and of micronuclei. Such a mechanism could conceivably lead to the presence in some roots of only a single cell with a particular aberration. It cannot, however, be held to alter to any significant degree the frequency of cells with changed complements; most induced aberrant complements occur in several cells in a root, and the majority of cells in most roots appear to be normal.

THE NUMBER OF INITIAL CELLS

The cells from which a new root primordium is derived may be called 'primordium initials'. The evidence (e.g. Table 2, roots 25 and 4) indicates that, in the regenerated apex of an irradiated primary root, at least 9 primordium initial cells gave rise to the new primordium. When fully organized, and distinguishable, the primordium is made up of many cells; at this stage

the primordium from which root 25 developed may be said to have included 9 *types* of cells. In terms of numbers of cells, estimates of the absolute number of cells and of the number of types of cells within the primordium are very different and must not be taken to be the same. Thus a primordium may be said to have been derived from 3 primordium initials when it appears to contain 3 types of cells. But this does not seem to indicate that the fully formed primordium consisted of only 3 cells, or, more especially, that the actively growing meristem which develops from the primordium has only 3 meristem initial cells; rather it shows that 3 types of meristem initial cells are present (cf. Brumfield, 1943). If we accept the suggestion that, at 21 days after X-raying, the chimaerical roots are stable and have been so from the beginning, then the relative proportions of cells with normal and with changed complements would indicate that there was more than one cell with a normal complement among the primordium initials. The relative proportions of the various types of cells indicate about 3 normal cells to every 1 atypical cell. Therefore, where there are 8 abnormal cells, there should be 24 normal cells. The estimate of the number of initial cells now becomes 32 (on the same basis the number of primordium and meristem initials in lateral roots would be at least 20 (Davidson, 1959*a*). On the same basis, the number of meristem initial cells in the other unsplit primary roots fixed 21 days after irradiation may be estimated. Only one root (Table 2, root 11) appeared to have had less than 20 meristem initial cells, and the average number for the 8 unsplit roots is 24.

Estimates of the number of primordium initial cells are, of course, based on the observations of meristematic cells seen in mitosis many days after the primordium was formed. Strictly, these observations enable us to estimate only the number of meristem initial cells from which the meristem of the growing root is derived; but the minimum number of primordium initials may also be inferred since abnormal chromosome complements in the meristematic cells must have occurred in cells of the primordium.

The figure for the number of meristem initials in primary roots is considerably greater than the previous estimate (Brumfield, 1943) that the root of *Vicia* has 3 or 4 initial cells. But in those experiments the X-ray exposure dose was lower than that reported here, and, furthermore, the roots were prepared as sections. It is conceivable that other aberrations could have been present in the roots of the original experiment and yet were missed because the roots were sectioned.

The structure of the normal root of *Vicia* has been described with regard to the arrangement and mitotic activity of the cells (Clowes, 1959). Roots of *Vicia*, *Allium*, and *Zea* appear to be closely similar (cf. Clowes, 1959; Jensen and Kavaljian, 1958). In all these roots there are a large number of root initial cells lying over a cushion of mitotically inactive cells of the quiescent centre. Though it cannot be argued with any certainty that deductions valid for normal roots are valid for irradiated roots, or vice versa, nevertheless the agreement between the results from the two types of roots (i.e. that there are many initial cells) indicates that they are very similar in the pattern of their ontogeny.

It may be concluded that in regenerating primary roots after irradiation and probably in unirradiated primary roots of *Vicia*, there are at least 30 meristem initial cells from which the primary root is derived. It is important that this evidence is cytological, for the previous evidence (Brumfield, 1943) on cytological grounds cannot be reconciled with the recent results of studies of root structure (Clowes, 1956; Jensen and Kavaljian, 1958), yet it could not be ignored since it was obtained by the use of cell markers. The use of larger doses of X-rays may yet reveal that there are more than 30 initial cells.

The Pattern of Regeneration

Irradiated primary roots of *Vicia* grow at a greatly reduced rate and then remain stationary for several days before they begin to grow again. The period without growth is essentially a period in which there is no cell elongation; but it is probable that some cell division is occurring, otherwise split roots would not show the same chromosome aberration in both parts of the split root (Davidson, 1959*b*). This is also the period when the new primordium with its meristem is established. The new meristem that is formed within the root apex produces the new root growth. This new tissue is essentially an apical derivative of the old meristem in the way that lateral roots are derivatives of the meristem. The regenerated root has structural continuity with the original root, but does not, following irradiation, arise from exactly the same group of cells; of the original meristematic cells some die and chromosome changes are induced in others. More rarely, two or more new meristems are organized at the apex of the old root (Clowes, 1959) and branched roots are formed. The branches may contain similar aberrant chromosomes (Davidson, 1959*b*).

In the period of reorganization that leads eventually to the formation of new meristems and thus to new roots, the cells marked with changed chromosomes appear to become established as initial cells of the new root, and their descendents are found in division during later root growth. The possible long-term stability of the chimaerical structure of the roots would depend on the behaviour of the meristem initials and the quiescent centre; first it would depend on the rate of replacement of meristem initial cells by cells from the quiescent centre; second, on any differences in the chromosome complements of meristem initials and cells of the quiescent centre.

The Quiescent Centre

The quiescent centre is readily distinguishable because it is a fairly large group of identical cells situated at the apex of the mature root. It is most easily demonstrated because of its relatively low metabolic activity; cells of the quiescent centre take up little of the isotopically labelled compounds used to detect synthesis of DNA and proteins (Clowes, 1956, 1958, Hejnowicz, 1959) or carbohydrates (Rabideau and Mericle, 1953). Isolated single cells or a very small group of cells with properties similar to those of cells of the quiescent centre may occur in meristems but would be difficult to identify. In lateral

buds of *Tradescantia*, Naylor (1958), however, was able to demonstrate the presence of quiescent cells which contained the 2c amount of DNA (i.e. the amount in a diploid cell before synthesis). These cells could be stimulated to synthesize DNA and to undergo mitosis by reversing the apical dominance of the main shoot.

The present study has provided evidence of a large number of meristem initial cells. It does not show, however, that meristem initials are never replaced by cells of the quiescent centre and it raises several questions:

1. What is the relation between meristem initial cells and the cells of the quiescent centre?
2. What function does the quiescent centre fulfil?
3. What is the system in normal roots that establishes and then perpetuates a group of nondividing cells in the middle of a region of actively dividing cells, yet allows division of the dormant cells when the root has been damaged?

Solutions to these problems will considerably enhance our knowledge of cell growth and division within the meristem.

Summary

1. Viable atypical chromosome complements were induced in cells of primary roots of *Vicia faba* by X-irradiation.

2. The exposure dose of 600 r was delivered to the beans after 24 hours' germination, and the marked cells were seen in roots fixed 9, 11, and 21 days after irradiation.

3. In roots fixed at 9 and 11 days, up to 11 different chromosome complements occurred in a single root. In roots fixed at 21 days the maximum number of new complements in a single root was 8.

4. A root whose meristem contains 8 types of changed complements, together with the normal complements, must have at least 9 *types* of cells represented in its meristem initial cells. The relative frequencies of cells with abnormal and normal complements indicates that although there are 9 types of cells, there must be, at a minimum estimate, about 30 meristem initial cells.

5. Since 9 types of cells appear in the growing root, these must have been included in the primordium that is regenerated after irradiation. Thus, a primordium must have included at least 9 primordium initial cells.

6. These results indicate that the apex of the meristem of a growing irradiated primary root of *Vicia* can be estimated to be occupied by about 30 meristem initial cells.

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Life History and Reproduction of *Furcellaria fastigiata* (L.) Lam.

2. The Tetrasporophyte and Reduction Division in the Tetrasporangium

BY

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With two Plates and two Figures in the Text

Abstract

The tetrasporophyte of *Furcellaria fastigiata* (L.) Lam. is diploid and has 68 chromosomes. The plant is morphologically similar to, though slightly greater in stature than, the gametophytic plants, both of which have 34 chromosomes. Tetrasporangial initials appear in May and reduction division takes place in early November. The process of meiosis is basically very similar to that of higher plants. A modified iron alum aceto-carmine staining schedule gives satisfactory staining of the chromosomes of this plant.

Table of Contents

Introduction

THE development of the haploid nuclear phase and of the carposporophyte of *Furcellaria fastigiata* (L.) Lam. are described in an earlier paper (Austin, Pt. 1). This paper deals with the development of the second part of the diploid phase, i.e. the tetrasporophyte, and with the meiotic division of the large tetrasporangial nucleus. In addition, mitotic divisions in the haploid nuclei are described. The tetrasporangia were briefly noted both by Rosenvinge (1917) and Kylin (1923), but neither their development nor reduction

[1] This work was carried out in the Department of Botany, University College of Wales, Aberystwyth.

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division of their nuclei was described. It is believed that neither tetraspores nor carpospores of *Furcellaria* have previously been cultured in the laboratory.

The lack of critical cytological data for nuclear division in the Rhodophyceae as a whole is also evident (Drew, 1944; Bold, 1951). Many of the discrepancies between nuclear cytology of red algae and higher plants may be solely due to differences in technique and not of fundamental importance. Earlier workers, for example Lewis (1909), Svedelius (1911), Wolfe (1904), Yamanouchi (1906), and Kylin (1914), used chromic acid fixatives and sectioning methods. More recently, Westbrook (1935) has made observations on a number of Floridean nuclei and *Spermothamnion turneri* has been investigated by Drew (1934). In all these investigations fixatives containing formalin were used and these have certain limitations in cytological work. Apart from the work described here, only Rao (1953) working on *Polyides caprinus*, appears to have made use of modern squash techniques in studying the Rhodophyceae.

Materials and Methods

The source of the material used has been given in Part 1. Most of it was fixed in Carnoy's fluids (with and without chloroform). Formalin-acetic-alcohol (various combinations) and formalin-alcohol were also tried, both hot and cold. Of these, only Carnoy's fluids gave consistently good fixation for chromosome study, but the others were useful for material subsequently embedded, sectioned, and stained with Heidenhain's haematoxylin or Newton's gentian-violet method.

The appearance of the nuclei after fixation varies with the fixative used. Fluids containing formalin and chromic acid produce division figures invariably small in volume and often aberrant in appearance compared with material from an acetic alcohol fixative or from acetic acid alone.

The chromosomes were stained by a modification of the alum-carmine schedule described by Godward (1950). The degree of staining can be altered by carefully controlled mordanting with various concentrations of ferric ammonium sulphate and subsequent washing. Differentiation and destaining can be effected by immersing once again in the mordant bath. Repeated heating and cooling of the material in the aceto-carmine controls the intensity of the staining. The preparation must be thoroughly squashed to ensure spreading of the chromosomes, which are very small, as in most other red algae. Good results using these methods have already been obtained with other species (Austin, 1956; 1959). Feulgen staining worked well on this material, and supplemented and confirmed the figures obtained by the alum-carmine method, which gave rather more intense staining suitable for photography.

Stained preparations may be kept for 4 to 6 months if sealed with stiff glycerine jelly. They can be made permanent by a modification of McClintock's (1929) method or by the vapour exchange method (Bradley, 1948); the latter avoids the removal of the cover glass and subsequent interference with the distribution of the cells and nuclei.

Mitosis in the Gametophyte and Tetrasporophyte

Mitosis is best studied during March and April in the rapidly growing ultimate ramuli of the three types of plant. Material was fixed on the shore at low water and hourly from plants transferred to aerated aquaria.

All thalli showed actively dividing nuclei in the cells of the apical region where new cells were being cut off at the tips of the axial filaments. In addition, divisions were seen at the apices of lateral filaments and also in young cortical cells (which produce secondary laterals and hyphae). These latter nuclei (Pl. 1. 1, 2, 3) varied from 9μ to 19μ in diameter at prophase and were considerably larger than those of the apical cells; moreover, they were not obscured by dense granular contents.

During prophase the nucleus enlarged considerably and the chromosomes became visible as longer or shorter threads rather lightly and irregularly stained. These threads rapidly thickened or spiralized into elongate or dumb-bell shaped chromosomes, which for some time appeared to be interconnected. These could be counted at late prophase as 34 (in the gametophyte) or 68 (in the tetrasporophyte) highly contracted chromosomes lining the nuclear membrane and surrounding the very faint nucleolus (Pl. 1. 1, 2, 3).

At this point the nucleus reached maximum size and the nuclear membrane and nucleolus disappeared. The nucleus lost its shape and the chromosomes gathered together in a ring-shaped cluster which eventually resolved itself into a metaphase ring. Faint but definite spindle elements, apparently extra-nuclear and anastral, could be distinguished, and two very dense ring-shaped bunches of small chromosomes arranged themselves along the spindle and segregated to the poles at anaphase. The telophase nuclei moved far apart due to the elongation of the 'stem body' of the spindle and the oblique cross-wall formed long before the chromosomes despiralized into the resting condition.

Fixations were made at hourly intervals from evening low water to morning low water to include the period of high water at night. Some indication of a rhythm in mitotic activity was obtained. Maximum activity seems to be near the time of high water at night and not, as suggested for some other species, at midday low water (Rao, 1954, Thesis). Further observations over the whole lunar cycle are desirable to include a comparison with activity during neap tides.

The Place of the Tetrasporophyte in the Life History

In December and January haploid plants produce young male and female organs as described in Part 1 (Austin, 1960). On the female structures, in late January, the first diploid nuclear phase, namely the carposporophyte, begins to develop, and ripe carpospores are discharged from its gonimocarps during the following December.

The carpospores germinate to produce the tetrasporic plants which when mature have young tetrasporangia in May and release ripe tetraspores in mid-winter. Thus the ripe gametes, carpospores, and tetraspores are present at the

same time of year. Plants which have borne tetraspores can be distinguished in February and March by the remnants of fertile structures. Such plants frequently proliferate from their truncated apices where the rapid mitotic activity enables chromosome counts to be made with ease, particularly in nuclei of dividing cortical cells (Pl. 1), and the presence of 68 chromosomes confirms the diploid nature of the asexual tetrasporophyte.

Development of the Tetrasporangium

Sporangia are initiated in late April and become obvious in May. The sporangium arises sub-apically or apically on the distal end of an inner cortical cell, known as the sporangial mother-cell, near the base of a primary lateral (Fig. 1*a*). The nucleus of this cell migrates into the dense initial of the sporangium, where it divides, one daughter-nucleus passing back to the original position, whilst the other daughter-nucleus remains in the initial. The sporangium is then cut off by a concave cross-wall. Chromatophores can be observed in the sporangium from the beginning (Fig. 1 *a*, *b*).

Two sporangia may develop upon a single mother-cell, both to one side, or one on each side, of the apex. Occasionally two cells may be cut off, one distal to the other. The outer cell develops into the sporangium, whilst the inner cell remains undeveloped and poorly staining and has a small nucleus; it may represent the stalk cell which occurs beneath the tetrasporangia in many other forms.

Most of the sporangia are of about the same size and stage of development, but amongst the vast number present in late June, stages from a small protuberance to sporangia 40μ long with relatively large nuclei from 4μ to 8μ in greatest diameter (Fig. 1*b*) can be found.

Increase in size throughout August and September is accompanied by other changes (Fig. 1 *b–e*). The chromatophore becomes progressively subdivided until it is represented by small, more or less interconnected, ovoid or discular segments lining the whole of the sporangial wall (Fig. 1 *e*, *f*).

The sporangium increases in size up to a maximum length of about 110μ in November (Fig. 1*f*).

Meiosis in the Tetrasporangium

During early November the state of the sporangia in plants kept in large aquaria of aerated sea-water was examined daily until meiosis began. Material was then removed every 2 hours and fixed. The same process enabled mitotic divisions to be obtained in April from growing apices of both tetrasporophytes and gametophytes.

Material fixed between 4 and 12 November inclusive revealed nuclei in all stages of reduction division. The exact timing of this division is set out below.

On 14 November both in 1954 and 1955, the vast majority of sporangia were seen to be divided into four zonate spores. Rosenvinge, working on Danish material (1919), also records division during November, although he states that sporangia only begin to develop in August.

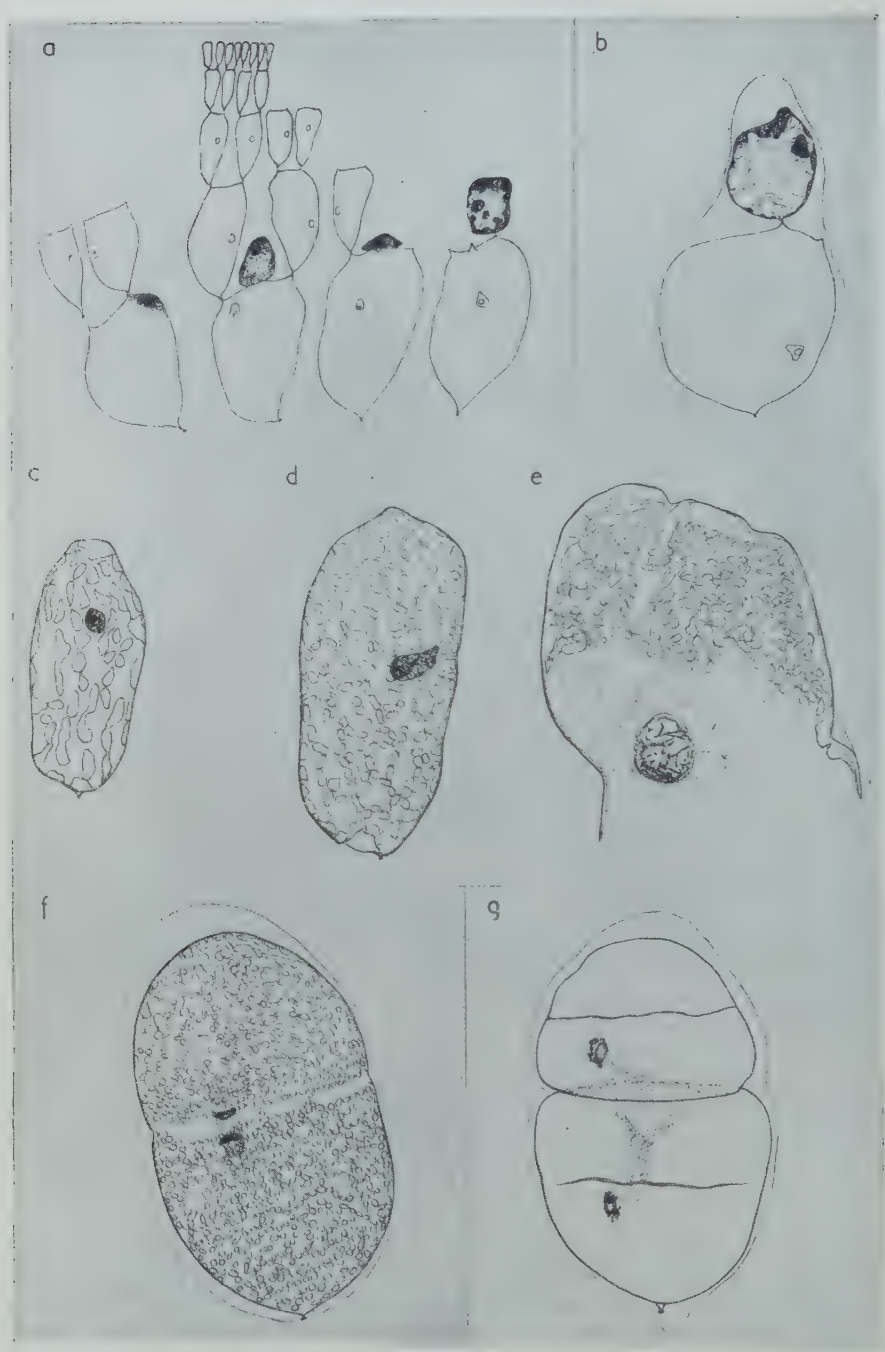

FIG. 1

From the present investigation it seems that meiosis in this species takes a number of hours to reach completion, perhaps 12 or more, but the process spread throughout the sporangia in an individual occupies several days. The event is highly localized in time within the annual cycle, recurring at almost the same time each year. Meiosis began a little earlier in 1955 than in 1954, but there was a difference in the dates of the tides, the first neap tides of November being also earlier in 1955.

The first changes in the nucleus marking the onset of division can be seen from 29 October to 5 or 6 November. The large nucleoli become vacuolate after fixation in fluids containing formalin.

As is usually the case, the earliest stages of prophase are not easily observed. A leptotene stage has been seen in only a few nuclei. The fine heterogeneously stained chromosomes are arranged in their extended state across the nucleus; the nucleolus and nuclear membrane are well marked.

The subsequent pairing phase seems difficult to interpret. A large number of nuclei of *Furcellaria* (Pl. 1. 4) have been seen in a state which has been variously called 'synapsis', 'synizesis', &c., by earlier workers. These were obtained with formalin fixatives and the figures probably represent badly fixed zygotene stages. In the better preparations using methyl-alcohol-acetic, a far less contracted nucleus with delicately pairing thread-like chromosomes is obtained (Pl. 1. 5).

The pairs can be easily distinguished and can be counted as 34 in better preparations (Pl. 1. 5). Coiling of one chromosome around its homologue can be made out in many instances (Fig. 2*a*; Pl. 1. 5, 6). The nucleolus is conspicuous but it is difficult to distinguish the nucleolar chromosomes, although two or three of the pairs appear associated with the nucleolus.

The chromosomes have contracted little up to and including the pachytene stage, and vary from 2·5μ to 9·5μ in length. The nucleus varies from 11μ to 37μ with nucleoli from 8μ to 13μ in greatest diameter.

Attraction between homologous pairs now decreases and this, together with the position, number, and behaviour of the chiasmata, results in the formation of typical diplotene configurations (Fig. 2*a*; Pl. 1. 6–9). The bivalents at this

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Fig. 1. Development of the tetrasporangium:

(*a*) Various stages in the origin of the tetrasporangial initial, apically and subapically, upon cells of the middle cortex, ×290;

(*b*) the appearance of the young tetrasporangium in early June; the chromatophore is dense and most conspicuous towards the upper (distal) part of the cell and the wall or sheath is well marked, ×460;

(*c*, *d*, *e*) stages in the enlargement and development of the sporangium from June to November; the subdivision of the chromatophore and enlargement of the nucleus is evident in figs. *b*–*e* (*c* and *d*, ×460; *e*, ×725);

(*f*) a mature tetrasporangium in early November with nucleus in first meiotic anaphase; the separation of the lining layer of chromatophores and the beginning of the cross-septum can be seen, ×420;

(*g*) a mature tetrasporangium with the nuclei in second meiotic prophase, the rudiments of the cross-walls in each half-sporangium can already be seen, ×420.

stage show that their centromere regions are mostly submedian or median and the number of chiasmata are mostly two (Fig. 2*e*). A few bivalents have three and one or two have one chiasma. It is possible that lack of association occurs in one chromosome pair, and the appearance of univalents in the diakinetic figure has added to the difficulty of establishing the chromosome count.

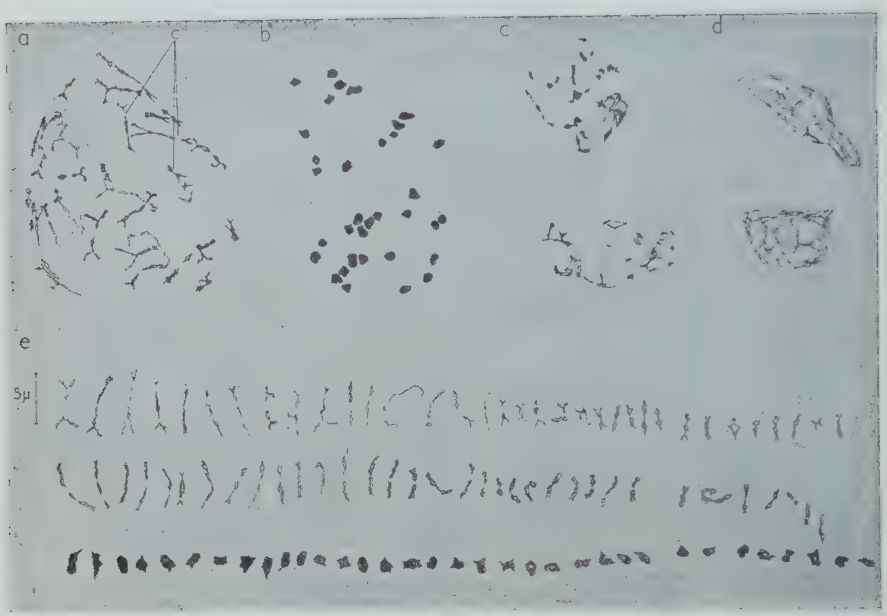

FIG. 2. Meiosis in the tetrasporangial nucleus:

(*a*) pachytene; four strands can be distinguished in some bivalents, but centromere attraction persists; this structure (*c*) can be seen in most of the bivalents, × 1,400;

(*b*) 'early'-diakinesis; the nuclear membrane disappears and the highly contracted bivalents begin to cluster towards the centre of the nucleus around the nucleolus, × 1,400;

(*c*) late telophase of the first meiotic division; the nucleolar membrane and nucleolus have reconstructed, × 1,000;

(*d*) interphase between the two divisions of meiosis, with the nuclei in a near resting condition, × 1,000;

(*e*) chart of the 34 bivalents as they appear at early diplotene, late diplotene, and diakinesis respectively. Incomplete association is seen in the 13th bivalent in the top row, × 1,400.

The diplotene phase is rather prolonged, the first part, as described above, being characterized by the extended nature of the chromosome pairs which assume typical cross- and loop-shaped forms. These become distributed throughout the nuclear sap, giving the very large nucleus a pale, faintly stained appearance (Pl. 1. 7), which corresponds closely with the 'diffuse stage' described by other workers as occurring between synapsis and diakinesis in some algal nuclei (Westbrook, 1935).

The second part of the diplotene phase embraces spiralization and shortening of the chromosome threads. Their double nature becomes progressively obscured (Pl. 1. 8, 9). At diakinesis the pairs are represented by very short

thick almost globular bivalents varying from 0·9μ to 3μ in length, and which are easily counted (Fig. 2*b*, *e*: Pl. 2. 1).

The nucleolus is quite obvious at the end of diplotene and persists until the end of diakinesis which terminates prophase; at this time it begins to break up and the nuclear membrane vanishes. The bivalents previously lining the nuclear membrane or distributed throughout the nuclear sap collapse, firstly on to the clouded nucleolus (Fig. 2*b*) and then into an equatorial cluster around fragments of the latter (Pl. 2. 2). The nucleus has thus lost its prophase shape and the figure now has a very much smaller volume, varying from 18μ to 30μ in length with a width of 7μ to 12μ. This transient phase following diakinesis constitutes a clear prometaphase (Pl. 2. 2). At this time globular or irregular masses of nucleolar material (inferred from their staining) are present in and at the periphery of the division figures.

The chromosomes now arrange themselves briefly in a compact metaphase ring and begin to separate almost at once. Longitudinal hyaline striations, more obvious near the chromosomes, are clearly the spindle elements (Pl. 2. 3, 4). The poles of the figures are not well demonstrated with acetic-alcohol fixatives and no definite structures can be distinguished. Division is extranuclear and probably anastral (Pl. 2. 3, 4). The two sets of very highly condensed and somewhat clumped chromosomes separate to the poles as small ring-shaped clusters (Pl. 2. 4).

When the anaphase groups have separated from each other to almost the original length of the spindle the central part of the latter—the 'stem body'—elongates rapidly. Thus the two telophase nuclei are pushed into the upper and lower halves of the sporangium respectively. The first indications of the division of the sporangium into two halves are seen as early as anaphase or even metaphase and are described below under 'cytokinesis'.

During telophase of the first division, the ring-shaped clusters of chromosomes exhibit some clumping and occasional laggard chromosomes appear to confirm the suspected lack of association between a pair of the homologues.

Each daughter-nucleus assumes a saucer shape and the nucleoli appear again in the hollow sides which face each other (Naylor, 1958). The nucleolus at this stage appears to be closely associated with three or more of the chromosomes, but detail is difficult to resolve. A nuclear membrane forms and the nucleus increases rapidly in size. The clumped chromosomes despiralize and become more elongate and thread-like once more (Fig. 2*c*), the nucleus taking on a faintly staining appearance very like that of the 'resting state' (Fig. 2*d*).

Interphase is prolonged (cf. *Dasya arbuscula* (Westbrook, 1935), *Spermothamnion turneri* (Drew, 1934), *Polyides caprinus* (Rao, 1954)), and a large number of sporangia with their nuclei in this state are found (Fig. 2*d*); these nuclei are smaller than the original sporangial nucleus at prophase and are 15μ to 20μ and the nucleolus 5μ to 9μ in greatest diameter.

In the second division of meiosis the faintly staining heterochromatic regions of the 'resting' interphasic nuclei become rapidly condensed into short rather ill-defined chromosomes arranged around the periphery of the ovoid

nucleus. This constitutes the short prophase of the second meiotic division (Pl. 2. 5, 7). This is uncomplicated except by the tendency of the chromosomes to clump together and become indistinct, and resembles the ordinary mitotic prophase. These nuclei vary from 15μ to 30μ and the nucleoli from 6μ to 11μ in greatest diameter.

The conspicuous interphase nuclear membrane and spherical nucleolus disappear quite soon and the figure collapses into an irregular ring of very minute chromosomes. These become arranged into a small compact metaphase plate and second anaphase follows. The figures are small and probably poorly fixed, they are from 11μ to 17μ long and 4μ to 6μ broad.

The clumps of minute chromosomes separate during second anaphase and the 'stem body' of the spindle elongates to push the telophase nuclei into the upper and lower halves of each half-sporangium (Fig. 1*f*). These four nuclei now become reconstructed and pass into the resting state. Numerous nuclei like those in Plate 2. 6, 8 are seen in completely divided sporangia, and it seems that the chromosomes retain their contracted form whilst the nuclear membrane and nucleolus become reorganized. The chromosomes finally despiralize and assume the 'resting condition' in the nuclei of the maturing spores. The only change that takes place in the nucleus of such a spore between this time and its first mitotic division (upon the germination of the spore 5 weeks later) is that of growth in size.

Orientation of the spindles of the second meiotic division. It has been claimed for some species that the second metaphase spindles lie at right angles to each other. In *Furcellaria* the metaphase and anaphase spindle axes are arranged neither parallel nor at right angles to each other, but in a manner best compared with the opposite angles of a tetrahedron. Later, when separated by the newly formed cross-walls, the four nuclei lie for some time with the first and third at a higher plane of focus than the second and fourth and in a marked zigzag. This arrangement can be accounted for if regarded as the persistence of the tetrahedral arrangement of anaphase spindles.

It has been thought that the tetrahedral arrangement is primitive. If this and the above observations are true it may be that the nuclei arrange themselves for a short time in the tetrahedral manner before migrating to their more or less linear or zonate position and that the transition marks some sort of relationship between the two spore arrangements.

Cytokinesis or cell division. The first indications of this appear at early anaphase I, when a circumferential hyaline band is seen round the middle of the sporangium. This is caused by the annular deposition of wall material parting the lining mass of small chromatophores (see page 299 and Fig. 1*f*). The edge view of the ingrowing septum can be seen as a refractive undulating transverse band (Fig. 1*g*). Later this establishes contact with an ill-defined transverse plate produced from the central portion of the 'stem body'.

The second cross-wall begins to form when the two interplase nuclei are in late prophase II (Fig. 1*g*). The same sequence of events takes place and results in the sporangia being divided into four spores arranged in a linear or

zonate manner. These round up a little and separate from each other slightly after the splitting of the first formed septa. The thick hyaline wall layers are laid down, but the spores remain attached to each other by pit connexions. In the spores the chromatophores become increasingly pigmented, causing the fertile ramulus, which has become progressively darker since August, to become deep red-brown.

About 5 weeks pass between the time of meiosis and the time the tetraspores are ready for liberation, in mid-December. During this period the spores enlarge, the cortical cells surrounding them become less and less starchy, and their gelatinous cell walls appear to increase in thickness. These processes result in an increase in girth of the fertile spore-bearing ramuli.

Finally the tetraspores are shed; they are slightly smaller than the carpospores and many more are produced, between 1 and 2 million in a plant of average size. At first the four spores are squeezed out together through tiny superficial ruptures and tend to adhere together by means of the copious mucilage produced by the breakdown of subsurface cells and exuded with the spores. Following the exudation of more spores, the whole surface of the ramulus wrinkles, cracks, and peels away, exposing more and more spores. Between 4,000 and 5,000 spores can be exuded from the surface of a discharging tip in a matter of minutes.

A long gelatinous core of medullary filaments is left and persists for some time, but is eventually shed, leaving the ramulus truncated. In three to six weeks these ends are seen to have hardened and to have produced short papillae which are rudiments of new ramuli, and which elongate throughout the growing season; they rarely produce spores the same year, but can become fertile in the following season. Often, however, the whole frond which has been fertile becomes detached and lost, perhaps because of lack of vigour or due to the weight of epiphytic growths.

Early Stages in the Germination of Spores

Since the season of liberation of both types of spores, their early development, and the method used in their investigation were similar for tetraspores and carpospores, both will be considered together.

The peak period of spore discharge is of short duration, occurring approximately between 26 December and 8 January (Austin, Pt. 1). It is thus only possible to obtain plants with perfectly ripe spores, in nature, on one spring tide period during the whole year. However, in addition to gathering plants at this time, near-mature specimens were collected at the previous spring tides and placed in the laboratory in running sea-water.

It was found that the most satisfactory method for 'sowing' spores on to a suitable substrate was by suspending fertile fronds, selected for freedom from epiphytic growth, near the surface of the water in large aquarium tanks, on the bottom of which the pieces of material upon which the spores could settle were placed. These included glass slides, smooth and ground; white tile, glazed and unglazed; fragments of earthenware and pieces of rock taken from

the sea-shore. Gentle agitation by bubbling air for 24 hours ensured that the discharged spores were well distributed in the tanks and would settle more or less uniformly when the agitation ceased.

At least 2 days were necessary to allow the spores to become attached more or less firmly to the pieces of substrate which were then transferred to flat-bottomed glass tanks of 6–12 litres capacity. A gentle flow of sea-water circulated through the tanks, and they were placed under different intensity of illumination. This was done (i) by painting the outsides of some tanks and covering them with slate tops (giving an illumination of *c.* 0–5 mc.); (ii) by placing other tanks in deep shade (*c.* 20–50 mc.), whilst (iii) the remainder had full light from fluorescent strip lighting close overhead (*c.* 800 mc.). The temperature was at all times no more than 1° C. above that of the sea. The carpospores and tetraspores were, of course, cultured in separate tanks.

In addition to growth in flowing sea-water, the following culture media were used: (i) Filtered sea-water; (ii) Sterilized filtered sea-water; (iii) A series made up both in filtered sea-water and in sterilized sea-water with nutrients added as recommended by Kylin (1917), Harries (1932), and Føyn (1934).

In order to grow the spores in their natural habitat but at the same time to have them available for examination in the laboratory, the following method was devised. A smooth concrete mixture was poured into rectangular moulds (*c.* 35×25×10 cm.) and when partially set pieces of plate glass (smooth and frosted), tile, rock, and brick were pressed into the surfaces to leave a permanent impression. When the mould had set hard the pieces fitted closely into the cavities so formed. The pieces were then scrubbed clean and sterilized and placed in tanks where tetraspores or carpospores were allowed to settle on them.

After a few days these pieces of substrate with spores attached were taken to the shore in a bucket of sea-water and fitted into their respective cavities on the concrete moulds already in place in a lower littoral lagoon where the species normally grows.

The moulds weighed between 8 and 12 lb. (4–5 kg.) and retained their position well over periods of more than 9 months, though some of the pieces of substrate which were not bedded deeply enough were dislodged. The pieces were periodically examined in the laboratory and returned to their moulds.

Germination both of tetraspores and carpospores took place both in the flowing sea-water and in cultures of filtered sea-water as well as in cultures with nutrients added according to Harries (1932). However, there was a very high mortality rate amongst the spores although some appeared to remain healthy but undivided for up to 8 or 9 weeks (cf. *Polyides caprinus*: Rao, 1956). Mortality increased with increase in light intensity, and contamination by bacteria and ciliates appeared to add to this. Antibiotics were not used. The sporelings lived longest in the lowest light intensities if contamination by ciliates was kept down; none lived for more than 4 months.

The spore flattened where it touched the substratum and the gelatinous

investment may have helped to attach the spore. Germination began usually in the first 5 days with the appearance of an oblique cross-wall which was usually orientated parallel to the direction of incident light. Other walls then arose parallel to the first and then septa oblique or at right angles to the latter resulted at the end of 10–25 days in a sporeling consisting of an ovoid mass of small cells. The chromatophores were arranged chiefly in the peripheral lining layer of cytoplasm and, together with the starch grains, distributed themselves into each product of division.

The rate of development was slow compared with that of other Floridean spores such as *Porphyra umbilicalis* (Drew, 1954) and *Gracilaria verrucosa*, *Lomentaria articulata*, and *Gigartina stellata* (Jones, unpublished). However, Rao (1956), using *Polyides caprinus*, also obtained similar slow rates of development of sporelings. The development of the carpospore into an ovoid cushion of cells closely resembled that of the tetraspore.

Spore growth in natural conditions. The spores did not retain their attachment very successfully except on the rough earthenware and unglazed tile. Their settlement under artificial conditions was probably incomplete and therefore easily destroyed by the scouring action of sand over the surface of the blocks. Even those which were attached for a period and began to grow were finally dislodged and their places taken by diatoms and sporelings of *Enteromorpha* and other algae; it may well be that small but deep fissures and/or the presence of other organisms such as bacteria and diatoms and perhaps larger sheltering species may be conducive to successful germination and establishment of sporelings of *Furcellaria*.

It seems clear that, in nature, development of both types of spore is well under way before the beginning of February and, like sporelings of many other species of red algae, appear unchecked by the winter conditions. The period of rapid growth in plants of *Furcellaria* begins very early in the year (Austin, 1960). It appears thus that both sporelings and adult plants are adapted to growth in the winter conditions prevalent in our latitudes. Further developments of the above described method of keeping spores and sporelings under observation on the sea-shore might yield useful results.

Discussion

The life history of *Furcellaria fastigiata* has already been discussed at some length (Austin, 1960; Pt. 1). The chromosome counts made in the present investigations show that both gametophytic plants are haploid and that the carposporophyte and tetrasporophyte are diploid. The life history therefore seems to be cytologically diphasic and morphologically triphasic (Drew, 1944).

Some recent workers (Rao, 1956; Drew, 1944, 1955) have indicated that the early belief that the haploid number of chromosomes for most of the diplobiontic red algae was 20 cannot be accepted. Indeed the high number demonstrated in *Polyides caprinus* (Rao, 1956) together with preliminary investigations carried out on other Florideae (Austin, 1955) suggest that the

chromosome numbers in this class may vary considerably from species to species.

The fixation of the nuclei of red algae is difficult and improved methods have yet to be evolved. Probably the gelatinous and cartilaginous thalli impede penetration of fixative fluids and the physiological condition of the plant may well be disturbed immediately it is removed from the sea. Nevertheless, the techniques used in the present investigation indicate that mitotic and meiotic division differs only in certain details from that found in higher plants. It is possible that in this species and perhaps in other red algae synapsis of the chromosomes occurs quite early; the chromosomes then pass through rather prolonged pachytene and diplotene with little further contraction or spiralization. In early diplotene the formation of typical 'open' looped and cross configurations, together with the distribution of these latter around the periphery of the now very large nucleus, produce a figure which stains weakly and appears lacking in chromatin. Then rapid spiralization of the extended bivalents occurs until they reach their smallest volume at metaphase and anaphase. This delayed spiralization or condensation of the chromosomes might be a possible explanation of the 'diffuse stage' described in the meiotic divisions of a number of red algae by earlier workers.

Acknowledgements

In conclusion I wish to express my thanks to Professor L. Newton for her constant counsel and encouragement throughout the investigation. In addition, I am grateful to Professor P. T. Thomas and Dr. K. Lewis for their guidance concerning cytological techniques and to Dr. D. J. Crisp and Dr. M. T. Martin for many useful criticisms during the preparation of the manuscript for publication.

Summary

1. Chromosome counts from growing apical cells show the tetrasporic plants to be diploid and it must be assumed they develop from the carpospores since these are also diploid.

2. The development of the tetrasporangium occupies the period from May to November. Reduction division then occurs synchronously in all sporangia to produce four haploid zonate spores.

3. Modifications of an iron-alum aceto-carmine staining technique have been used to demonstrate the chromosomes at mitosis and meiosis.

4. Meiosis in *Furcellaria* exhibits few of the peculiarities described by previous workers as occurring in other red algae. Nuclear divisions were observed in a number of other common Florideae and were seen to resemble closely the sequence of events in higher plants.

5. A definite spindle is present and this is extra-nuclear and probably anastral. The haploid number of chromosomes, counted during the well-marked late diplotene and diakinesis, is 34.

6. Tetraspores are liberated as a result of disintegration of the fertile regions of the thallus proceeding from the surface inwards.

7. In the laboratory germination of both tetraspores and carpospores, liberated at the same time of year, is poor.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Late mitotic prophase in the nucleus of a dividing cortical cell of the tetrasporophyte. The $2n$ number of 68 chromosomes can be counted. (×1,600)

Figs. 2 and 3. A late and an early stage in mitotic prophase of nuclei in dividing cortical cells from a female and male tetrasporophyte respectively. The $2n = 34$ number of chromosomes can be seen in each case. (×3,000)

Figs. 4–9. Meiosis in the tetrasporangial nucleus (all approx. ×1,600).

Figs. 4 and 5. Early prophase nuclei in which pairing is taking place and the haploid number of threads can be seen. Clustering of the chromosomes to one side of the nucleus in Fig. 4 may be due to poor fixation. A more typical zygotene is shown in Fig. 5.

Figs. 6 and 7. Early diplotene figures. Centromere attraction has diminished and the homologous chromosomes begin to separate to form open configurations. In (7) this process has reached a maximum and the 'open' looped and crossed bivalents line the nuclear membrane of the now very large nucleus. This figure closely resembles the 'diffuse stage' described in some red algae.

Figs. 8 and 9. Later diplotene—nuclear volume decreases and the condensation of the chromosome pairs proceeds rapidly.

Plate 2

Figs. 1–8. Meiosis in the tetrasporangial nucleus (all approx. ×2,000).

Fig. 1. Diakinesis—the nucleolus which has a beak is breaking up and the nuclear membrane is only partly present.

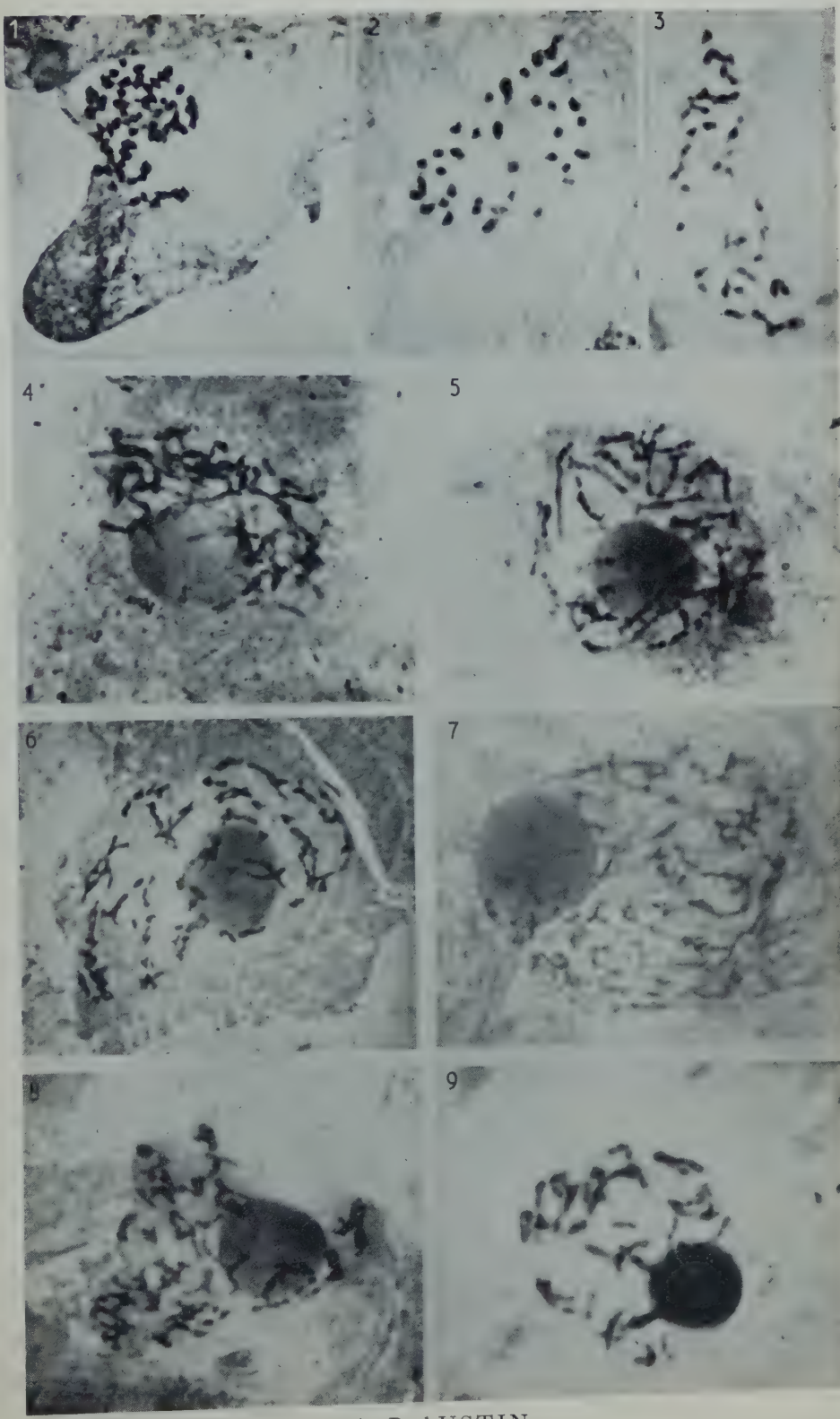
Fig. 2. Prometophase—a rather irregular equatorial assemblage of the chromosomes after the nucleolus and nuclear membrane have vanished, and before the spindle has organized. A certain amount of clumping of the bivalents has occurred.

Fig. 3. Early anaphase—disjunction is in progress, but the highly condensed and rather clumped chromosomes make the resolution of detail difficult.

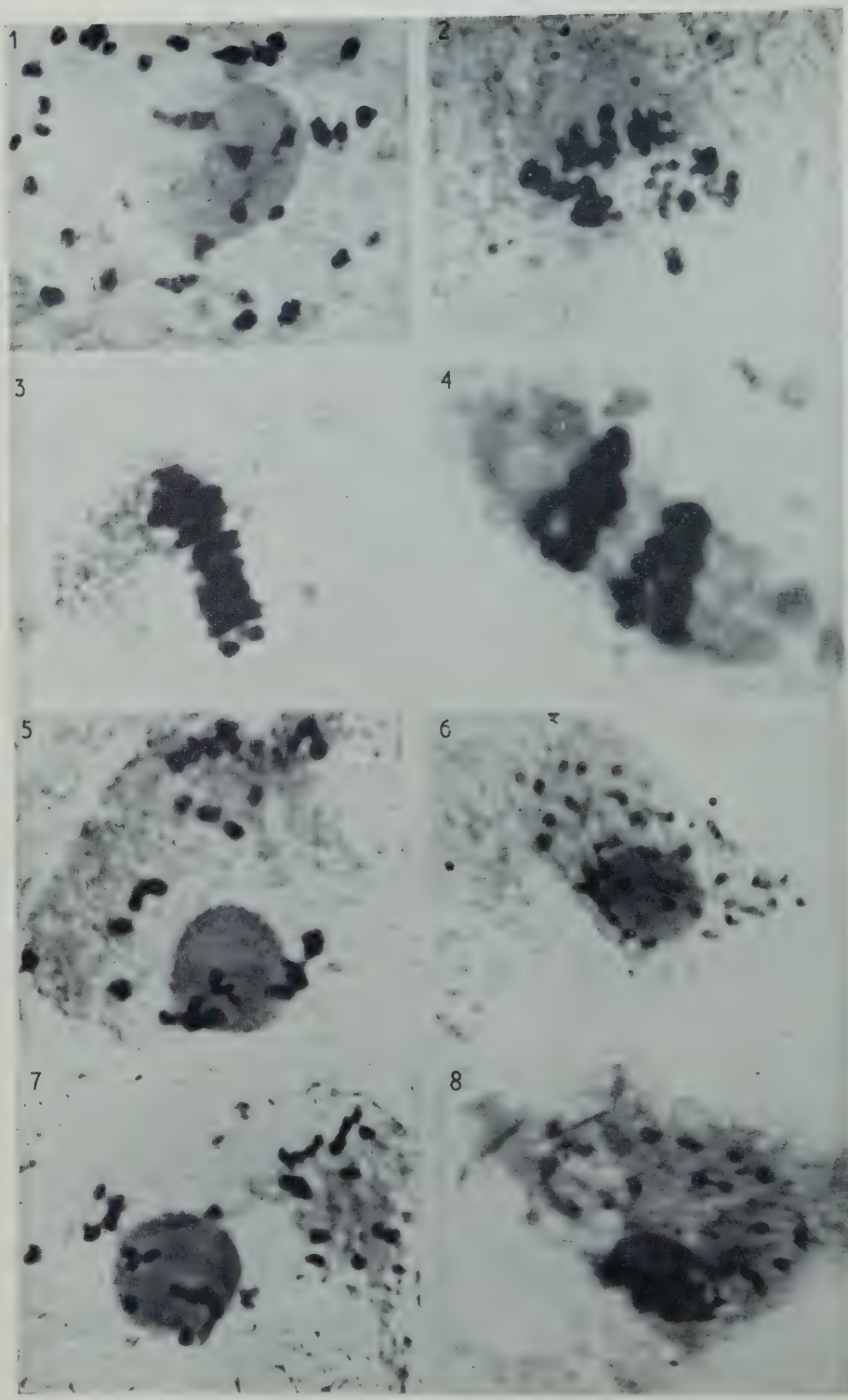
Fig. 4. Late anaphase—the two ring-shaped groups of tiny chromosomes were apart on recognizable spindle elements.

Figs. 5 and 7. The two daughter nuclei of the first meiotic division now in prophase of the second division. They are little more than half the size of the late prophase nuclei of the first division.

Figs. 6 and 8. Reorganization of two of the telophase nuclei after the second meiotic division. The nucleoli and nuclear membranes have appeared but the chromosomes appear to retain their condensed state for some time.



A. P. AUSTIN



A. P. AUSTIN

Effects of indoleacetic acid, naphthalene-acetic acid, and kinetin on phosphorus fractions in hypocotyls of dwarf bean (*Phaseolus vulgaris*)

BY

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With two Figures in the Text

SUMMARY

Total phosphorus and orthophosphate in hypocotyls of dwarf bean were increased by NAA and unaffected by kinetin. Acid-soluble bound P, representing several different compounds, was increased by NAA and by higher concentrations of kinetin. Ribonucleic acid phosphorus was greatly increased by NAA alone and slightly inhibited by kinetin (1 mg./l.). The large effect of NAA is probably associated with increased cell number.

Kinetin strongly inhibited formation of roots in bean hypocotyls, apparently not by inhibiting cell division, but by influencing the kind of cell produced.

INTRODUCTION

SEVERAL cytological investigations have shown that auxins influence the metabolism of nucleic acids, particularly kinetin (6-furfurylaminopurine). Skoog and Miller (1957) have pointed out the role of kinetin and indoleacetic acid (IAA) in desoxyribonucleic acid (DNA) synthesis connected with mitosis and cytokinesis. Naylor, Sander, and Skoog (1954) demonstrated cytologically that variation in the balance between indoleacetic acid and kinetin may determine the types of growth and organ formation. The evidence for this kind of relationship is mainly cytological, but the present paper deals with quantitative changes in phosphorus fractions, especially DNA and RNA, in material undergoing active growth.

A less detailed investigation of intact tomato plants by Andrejewa and Morozowa (1959) showed that treatment with IAA increased total phosphorus and nitrogen contents.

MATERIALS AND METHODS

When the hypocotyls of young dwarf bean plants severed a few centimetres below the cotyledonary node are kept with their bases in culture solution, adventitious roots form on the hypocotyl and sometimes on the epicotyl. This process is greatly stimulated when the hypocotyls are pretreated for some hours with indoleacetic acid (IAA) or naphthalene-acetic acid (NAA). Production of roots was used by Luckwill (1956) to assay growth substances in the presence of growth inhibitors; the number of roots formed was, within limits, proportional to the amount of growth substance supplied. Hemberg (1951, 1954) and Gorter (1958) used this region of the bean plant to study

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factors concerned with root formation, particularly boron. The stimulation of cell activity by auxins in this material makes it suitable to investigate the effects on phosphorus fractions.

Plants of dwarf bean (*Phaseolus vulgaris* var. Canadian Wonder) grown in John Innes compost were used 2 weeks after germination when the primary leaves were fully expanded and the first internode was beginning to elongate. Each plant was cut exactly 4 cm. below the cotyledonary node and the surface of the hypocotyl was sterilized by dipping for a few seconds in hydrogen peroxide (20 vol.); the hypocotyl was washed in water, kept for 24 hours in aqueous solutions of IAA or NAA in the shade, and then placed with the hypocotyl dipping in quarter-strength Hoagland solution supplemented with trace elements. Control plants were kept for 24 hours in distilled water before being placed in the nutrient solution. Some experiments tested the effect of adding kinetin to the culture solution. At the time of sampling, 1 week after excision, roots had not usually erupted through the epidermis, but the treated hypocotyls were always more swollen than the controls. The 4-cm. lengths of hypocotyl were cut off and washed with distilled water, dried with blotting-paper and weighed. The phosphorus (P) compounds were divided into four fractions: (1) acid-soluble bound P and orthophosphate, soluble in cold 0·2 *N*–$HClO_4$; (2) Lipid P, soluble in ethanol-ether (3:1); (3) Ribonucleic acid P, soluble in *N*–$HClO_4$ on soaking overnight at room temperature; (4) Desoxyribonucleic acid P, soluble in *N*–$HClO_4$ on incubating at 37° C. overnight as described by Holden (1952), see Table 1.

TABLE 1

Effect of IAA *on Phosphorus Fractions* (*μg. per hypocotyl*)

	Concentration of IAA (mg./l.)			
Phosphorus extracted by:	0	5	10	20
1. 0·2 *N*–$HClO_4$:				
acid-soluble bound P	35	95	62	23
orthophosphate	232	380	243	225
2. Ethanol-ether (3:1):				
Lipid P	64	130	119	82
3. *N*–$HClO_4$ room temperature overnight:				
RNAP	31	48	36	38
4. *N*–$HClO_4$ 37° C. overnight:				
DNAP	9	11	10	9
Total	371	674	470	377

RESULTS

(*a*) *Relative effects of* IAA *and* NAA

As a guide for subsequent experiments the relative effects of IAA and NAA on change in fresh weight and total P were compared with time after pretreatment (Fig. 1). The fresh weight of the controls and of the hypocotyls with the lowest concentration of growth substance (0·1 mg./l.) decreased at first but treatment with stronger solutions (10 mg./l.) increased fresh weight

for seven days, NAA having the greater effect. The total P content per hypocotyl also increased up to 7 days: here also NAA was more effective than IAA, increasing the phosphorus content from 200 to 500 μg. per hypocotyl. Twenty mg./l. NAA was after found to give a slightly larger response than 10 mg./l. and this concentration was used in most experiments.

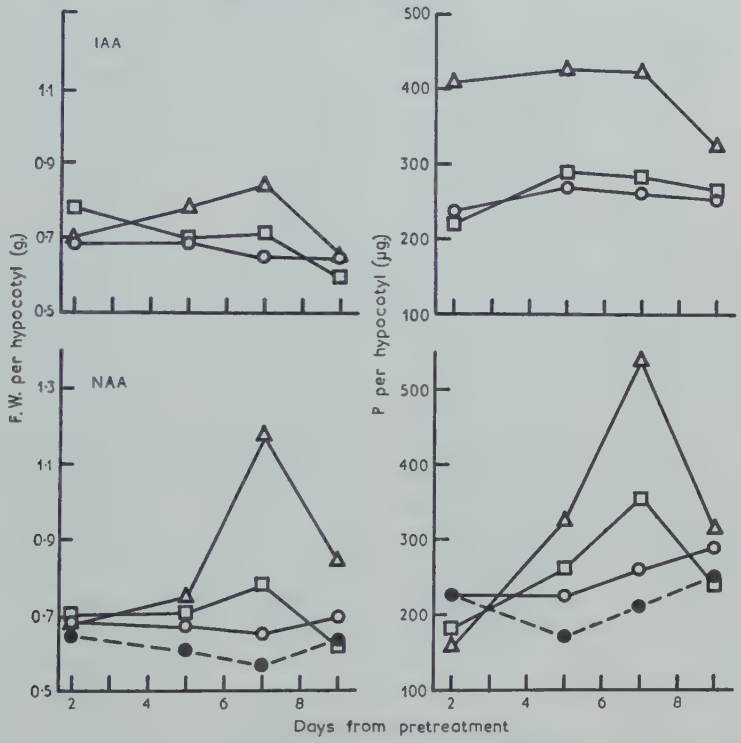

FIG. 1. Changes with time in fresh weight and total phosphorus content of a 4-cm. length of bean hypocotyl after pretreatment with IAA or NAA. ●- - -● control, o—o 0·1 mg./l., □—□ 1·0 mg./l., Δ—Δ 10·0 mg./l.

The amount of phosphorus absorbed by the hypocotyls in a week varied between experiments, apparently depending on the weather both before and after the hypocotyls were excised and was particularly affected by the degree of wilting before placing in nutrient solution. If wilting was severe, recovery was correspondingly slow, and less phosphorus was absorbed.

(*b*) *Effect of concentration of* IAA *on phosphorus compounds*

Because of the variability between experiments the concentration of growth substance that gave maximum response could not be established precisely. In one experiment phosphorus uptake was greater with IAA at 5 mg./l. and the P contents of all the phosphorus fractions were highest at this concentration; the highest concentration of IAA tested (20 mg./l.) had no effect on P uptake.

(*c*) *Effect of pretreatment with* NAA *and subsequent treatment with kinetin on phosphorus fractions.*

These experiments were of factorial design, testing presence or absence of

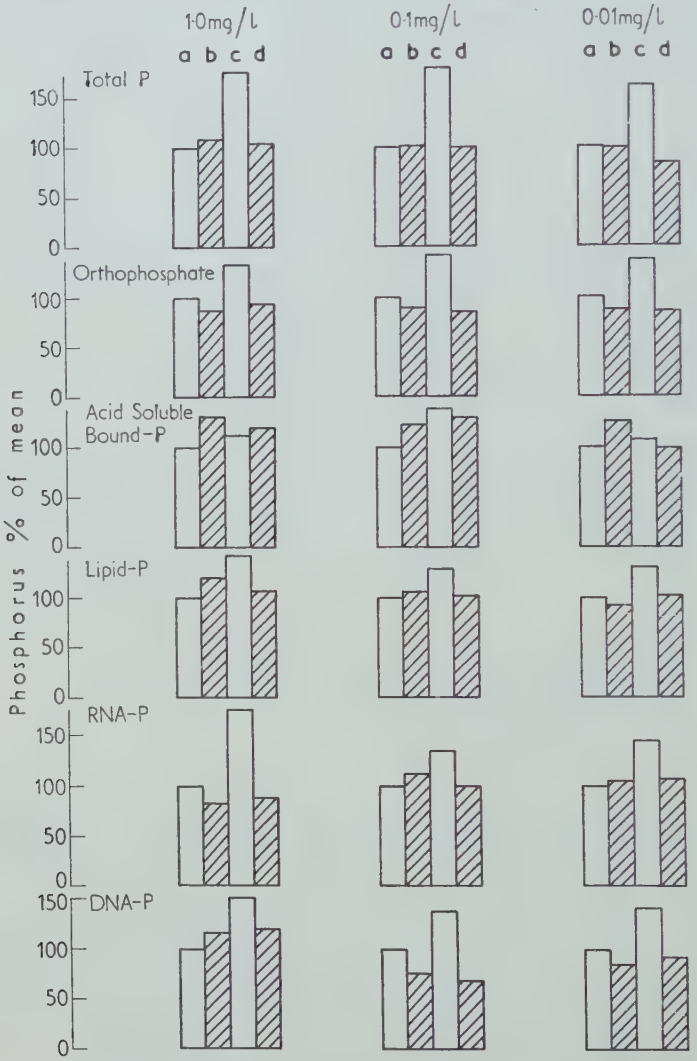

FIG. 2. Changes in phosphorus fractions of bean hypocotyls treated with naphthalene-acetic acid (20 mg./l.) and kinetin in factorial combination. Results expressed as per cent. of mean. *a* = mean. *b* = −NAA+kinetin *c* = +NAA−kinetin *d* = +NAA+kinetin.

NAA pretreatment (20 mg./l.) and subsequent kinetin treatment (1·0, 0·1 or 0·01 mg./l.). Fig 2 shows changes in phosphorus fractions as a percentage of the mean plotted in the form of histograms.

Total phosphorus was unaffected by kinetin and greatly increased by NAA.

Orthophosphate was considerably increased by NAA but only in the absence of kinetin; it was decreased by kinetin. By contrast, *the acid-soluble bound phosphorus* was increased by both by NAA and by the highest and medium concentrations of kinetin. *Lipid phosphorus* was increased by NAA and 1 mg./l. kinetin given separately, but together these had no effect; the other concentrations of kinetin were ineffective. *Ribonucleic-acid phosphorus* was greatly increased by NAA given alone but not with kinetin; 1 mg./l. kinetin, but not the lower concentrations, decreased it slightly. *Desoxyribonucleic-acid phosphorus*, on the other hand, was increased by 1 mg./l. of kinetin and also by NAA but the other concentrations of kinetin decreased it. The opposite effects of kinetin on DNA and RNA contents are shown in Fig. 2.

Discussion

The bound phosphorus of the first fraction almost certainly represents several different compounds so that the rather large changes produced by IAA or NAA and kinetin are difficult to interpret. The orthophosphate content is increased by NAA and slightly decreased by kinetin, and total phosphorus uptake is unaffected by kinetin, so we suggest that the rate at which absorbed orthophosphate is used is increased by kinetin. Little is known about the nature of the effect of growth substances on lipid compounds and the increase both by NAA and kinetin in the higher concentrations cannot be explained.

The large effect of NAA on RNA–P may be associated with increased cell number. Kinetin at 1 mg./l. inhibited RNA–P formation but the effect was small compared with the increase caused by NAA. Synthesis of nucleic acids (especially of RNA) and of protein appear to be connected. Olszewska (1959) found that kinetin accelerates incorporation of ^{14}C from sodium formate into RNA of the nucleolus and into the protein fraction of *Allium* root meristem. Gale and Folkes (1955) and Ýcas and Brawerman (1957) found other evidence for the mediation of RNA in protein synthesis.

There is evidence that kinetin inhibits root growth (Danckwardt-Lilliestrom, 1957) and de Ropp (1956). We found that 1 mg./l. of kinetin in nutrient solution inhibits root formation in untreated hypocotyls and greatly restricts root formation in hypocotyls treated with NAA. Kinetin apparently prevents the differentiation of roots, but does not always inhibit cell division; on the contrary, callus formation is stimulated, especially so in petioles, on which callus 8–10 mm. in diameter may be produced. Thus kinetin influences the kind of cell produced and the differences in phosphorus fractions reflect alterations in metabolism. Some observations on callus tissue formed on bean petioles by treatment with kinetin showed that the total phosphorus content expressed on the basis of fresh weight was only 50–70 per cent. of that of the petiolar tissue. This supports the suggestion that kinetin alters phosphorus metabolism.

Acknowledgements

We thank Miss Margaret Holden for helpful advice and criticism. One of us (W. M.-P.) is grateful for a Rockefeller Foundation fellowship.

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Aspects of Morphogenesis in a Dorsiventral Fern, *Pteridium aquilinum* (L.) Kuhn

BY

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With one Plate and ten Figures in the Text

Abstract

The formation of the horizontal dorsiventral rhizome of *Pteridium aquilinum* from the erect radial axis of the young sporeling is described. The shoot apex and the inception of leaves and buds at the apical meristem have been investigated, and their inception is shown to be essentially similar in long and short shoots, later differences being due to differing rates of growth and internodal elongation in the two types of shoot.

Introduction

ALTHOUGH in many ferns the axis is erect and of radial symmetry, dorsiventral main axes are also characteristic of a number of genera, these occurring in families not necessarily closely related, e.g. *Helminthostachys*, *Christensenia*, *Trichomanes*, *Davallia*, *Marsilea*, *Pteridium*, and *Polypodium*. Some dorsiventral species are epiphytic, e.g. tropical species of *Polypodium*; but others, like *Polypodium vulgare* and *Pteridium aquilinum*, are typical terrestrial forms. Bower (1923) held that the radial, erect habit was probably the primitive condition in the Filicales, the dorsiventral habit being a later and derivative condition.

Like other dorsiventral structures, the rhizomes of dorsiventral ferns have a single plane of symmetry: the leaves are borne on the upper side, usually in two rows, while the roots are formed predominantly on the lower side where, as in epiphytic species, the plant is most fully in contact with the substratum. But even in species like *Pteridium aquilinum*, in which the rhizome may be deeply immersed in soil or humus, a comparable dorsiventral habit is found. The dorsiventral habit is often, though not invariably, apparent in the arrangement of the vascular bundles within the stem. The morphology of these plants thus differs considerably from that of radially symmetrical ferns, though many of the underlying morphogenetic processes are evidently common to both.

The morphological and anatomical features of dorsiventral ferns have been described by a number of workers, from Hofmeister (1857, 1862) and Klein (1881, 1884) to Bell (1950 et seq.) and R. and C. Wetter (1954) among contemporary investigators. Although the features peculiar to dorsiventral ferns

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suggest many problems for the student of morphogenesis, few relevant investigations appear to have been carried out. Radially constructed ferns have been the subject of much analytical and experimental investigation by Wardlaw (1943 et seq.), Wetmore (1954), Cutter (1954 et seq.) and others, and it was felt that dorsiventral ferns could be similarly investigated with fruitful results. The study reported in this paper was carried out on *Pteridium aquilinum* (L.) Kuhn, a common dorsiventral fern. It has a creeping habit, leaves in two rows, and a number of additional features of interest, such as the mode of branching and the presence of long and short shoots growing at different levels in the soil.

Materials and Methods

Collections of *P. aquilinum* used in this investigation were made in various localities in Cheshire.

For direct observation of the shoot apex, the rhizome was cut off about 5 cm. behind the tip. The distal part was then placed upright, supported with plasticine, under a dissecting microscope magnifying 125 times. The scales were removed from the apex by means of a fine pair of forceps and sharp steel needles. Attempts were made to keep these rhizome apices alive on moist peat in covered pans in the laboratory for experimental treatment, but they could be kept alive for only a few weeks at most: the rhizome tips, especially those of long shoots, were very fleshy and rapidly became infected. Pieces of rhizome longer than about 5 cm., which might have survived for a longer period of time, were found to be unwieldy for experimental work. Moreover, because of the delicate nature of the apex, injuries due to any kind of surgical treatment spread extensively. For these reasons very few experimental observations could be made on apices of *P. aquilinum*, especially on apices of long shoots.

For observations of the young sporophyte, prothalli were grown in the laboratory. Spores were collected from ripe fertile fronds and were sown thinly on burnt leaf-mould in small pots which were placed in a trough of water and covered with glass plates. Growing prothalli were subsequently transferred to loam, on which they grew to maturity. Fertilization was facilitated by flooding the surface of the pots with water.

General Morphological Survey

Watt (1940) and Webster and Steeves (1958) have described the shoot system of *P. aquilinum*. It is made up of two main types of shoot, long shoots and short shoots, distinguished by the average lengths of the internodes, 30–40 cm. in long shoots and 0·5–2 cm. in short shoots. Between these there are all gradations of internode length, comprising the intermediate shoots. Each of these shoot types may arise from a shoot of the same type or either of the others as a branch, and a shoot may also change from one type to another during its growth. There is also a difference in the level at which the different shoot types grow—long shoots run in the deeper soil layers, and intermediate

and short shoots at successively higher levels, the average depth at which long shoots run varying with the type of soil. Observations made by the writer in the field are in agreement with Watt's description. On some wooded slopes in Cheshire, where the plants grew in decaying leaf mould, long shoots could sometimes be found at depths of only about 10 cm.

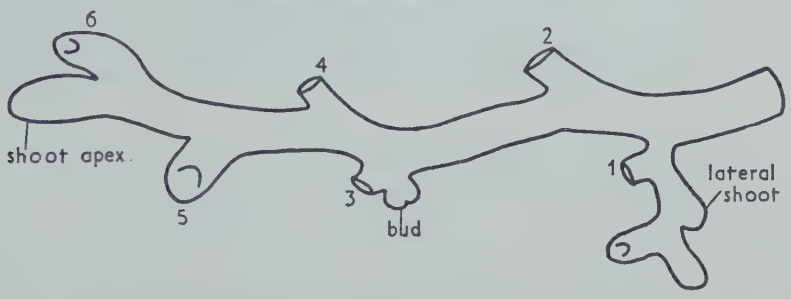

FIG. 1. Dorsal view of distal portion of short shoot. Leaves 1 and 3 have abaxial buds, that of leaf 1 having grown out into a lateral shoot. No buds are present at the other leaves. Note curvature of tips of leaves 5 and 6 towards the rear of the shoot. Roots not shown. ($\times\frac{7}{10}$)

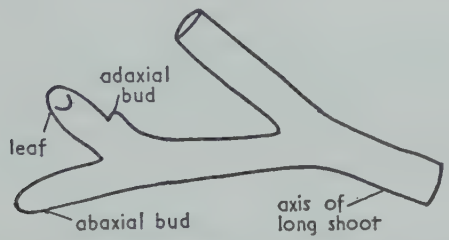

FIG. 2. Leaf on long shoot. The strong growth of the abaxial bud has pushed the leaf to a lateral position. ($\times\frac{2}{3}$)

Long shoots were characterized by a much faster rate of growth and internodal elongation than short shoots; the shoot apices, and buds formed on them, showing quicker growth.

A further difference, according to some observers, e.g. Webster and Steeves (1958), is that only the short shoots appear to bear leaves, each leaf having a bud at the base of its petiole, on the abaxial side (Fig. 1). Long shoots are said to bear no leaves, but only lateral branches. However, the present observations show that leaves are produced on long shoots. These, moreover, have two buds at the base of the petiole—the abaxial fast-growing bud, and a smaller slow-growing or dormant bud situated on the adaxial side, higher up on the petiole (Fig. 2).

Roots arise on all sides of the shoot, without any apparent regularity in their arrangement, and grow in all directions. In the radial young sporophyte, however, a single root arises beneath each leaf.

The shoot apex. The shoot apex is thickly covered with hairs. In long shoots the ventral side of the shoot apex projects, with the result that the

rhizome-end slopes strongly in a dorsal direction, the apical meristem being situated on this slope in a depression covered with hairs. In short shoots the distal end slopes to a much lesser extent, and the depression around the apical meristem is more pronounced than in long shoots. The shoot apex and the formation of the apical depression have been described by Hofmeister (1862), and Klein (1884) has described the apical meristem and the formation of leaf primordia. The apical cell is two-sided, with its long axis vertical (Plate, 1). The meristem itself is a blunt cone, more or less oval in outline and elongated laterally.

The Young Sporophyte

The young sporophyte is initially of radial construction, and has a three-sided apical cell (Plate, 2). It produces about 7 leaves in a space of about 3 months in the laboratory, the phyllotaxis being spiral. When this plant has produced about 9 leaves, a critical change takes place: the growth of the radial axis ceases, with the formation of two branches at the apex. These branches are dorsiventral, and grow downwards into the soil, to give rise to the shoot system of the adult plant. This phenomenon has been described by Hofmeister (1857), Bower (1923) and Gottlieb (1958), all these workers expressing the view that the axis of the young sporophyte undergoes a dichotomy, with the formation of two lateral shanks.

Observations. Fig. 3 shows the plan of the shoot apex of a young sporeling. The apical meristem has an approximately circular outline, and six leaves (1–6) are present, arranged spirally. Fig. 4 shows the apex of an older sporeling. The apical meristem has broadened out in a plane across that of the two youngest leaves P_1 and P_2. On the broadened part, on either side of the apical meristem and near its margin, a bud-like structure can be seen. Fig 5 shows a later stage. The two buds and the original apical meristem can be clearly distinguished. The position of the apical meristem can be ascertained from its relation to the younger leaves shown. One of the buds has formed a leaf. In Fig. 6 both buds have formed leaves, and the old apical meristem has become parenchymatous. In an older sporeling, shown in Fig. 7, the two buds are growing vigorously. They have turned in a horizontal direction, and the region between, i.e. the position of the old apical meristem, is occupied by a furrow formed by the rapid growth of the buds on either side. The two new shoots are dorsiventral. According to the writer's observations, then, the two new buds arise from meristematic tissue near the margin of the apical meristem, i.e. as buds are known to do in a number of other ferns, and true dichotomy is not involved—although the original apex may cease to grow. Later stages in bud formation (e.g. in Fig. 7) convey the impression of a dichotomy of the shoot apex, which probably explains the views of earlier writers, referred to above.

In the Plate, 3–5 are longitudinal sections of shoot apices of sporelings, illustrating the changes taking place, No. 3 being a very young sporeling. In the Plate, 4, the apical meristem has broadened laterally as described

above, and two buds are present in the shoot apex illustrated in No. 5, the section passing through only one of these medianly. This is a stage similar to that in Fig. 5.

Fig. 3

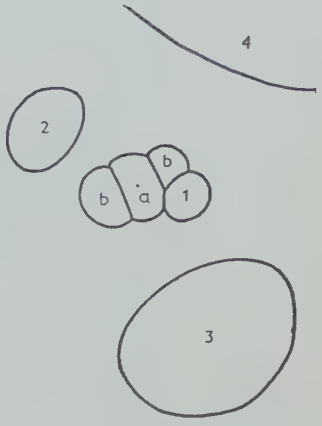

Fig. 4

Fig. 3. Shoot apex of sporeling. Note spiral phyllotaxis. *a*, apical meristem. (×60)
Fig. 4. Shoot apex of sporeling. The apical meristem has broadened out, and has formed two buds *b*, *b*. (×60)

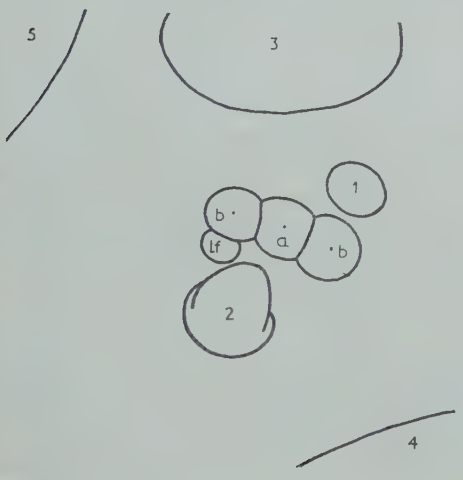

Fig. 5

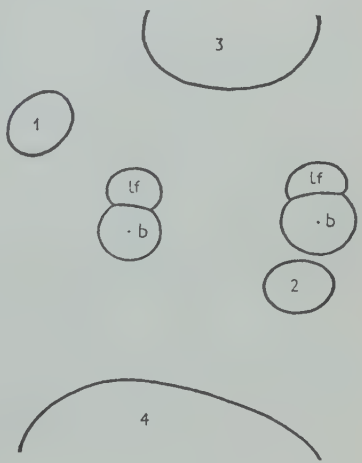

Fig. 6

Fig. 5. Shoot apex of sporeling. The bud on the left has formed a leaf *lf*. (×60)
Fig. 6. Shoot apex of sporeling. Both buds *b*, *b* have formed a leaf each. (×60)

The Formation of Buds and Leaves

The formation of buds and leaves and the mode of branching in the adult rhizome of *P. aquilinum* have received attention at the hands of many workers. Hofmeister (1862) stated that the main axis of the plant bore no leaves, but

branched alternately to the right and left, producing lateral shoots which bore leaves. He ascribed an adventitious origin to the buds on the petioles of these leaves. Büsgen (1915) interpreted the buds as branches produced by the rhizome, whose development was sometimes arrested by the growth of the leaves. He distinguished two kinds of shoots—short shoots which bore most of the leaves, and long shoots which had only a few leaves. According to

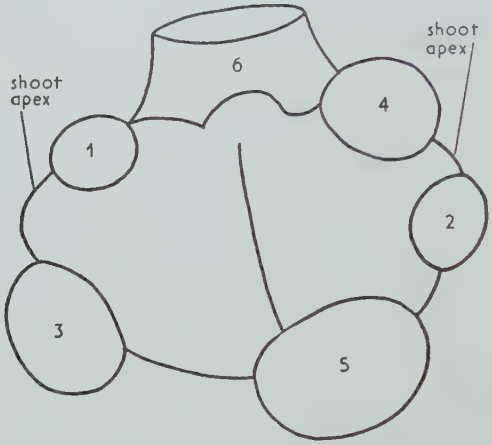

FIG. 7. Apex of sporeling. The shoot apices formed from the two buds are growing vigorously. The line down the middle represents the furrow between the two shoots. (×75)

Bower (1923), branching in *P. aquilinum* was by dichotomy, 'with unequal shanks: the shorter of these commonly bears a leaf at once, so placed that when mature the shank that bears it appears as a small bud at the base of the strongly developed leaf' (1923, p. 74). A number of other workers have expressed similar views, e.g. Tansley and Lulham (1904), Velenovsky (1905), and later Goebel (1928). Recently, Webster and Steeves (1958) have investigated the shoot system of *P. aquilinum*, and they, too, have stated that the main axis is leafless, leaves being produced only on short shoots.

Mettenius (1860) held the contrary view, that the petiole bore an abaxial bud and a weaker bud higher up on the adaxial side. If the leaf developed first, the buds appeared to be adventitious; and if the leaf and bud developed together, or if the bud grew faster and became like the main axis, a dichotomy appeared to have taken place, the leaf being produced by the lateral shoot. Mettenius cited a number of other fern species which resemble *P. aquilinum* in the placing of their buds, and Gwynne-Vaughan (1903) has described the somewhat similar instance of species of *Hypolepis*, where sometimes several buds arise from the base of each leaf.

The presence of two forms of shoot makes the mode of branching in *P. aquilinum* appear very complex, and the slow growth makes direct observation difficult. If branching is indeed solely by dichotomy, and if leaves are not produced by the long shoots, *P. aquilinum* would be fundamentally

different from all other ferns whose mode of branching is sufficiently known. For this reason, and since it is clear that most previous workers had examined only mature shoots, neglecting the actual site of organogenesis, the formation of buds and leaves in this species was re-examined by investigating the shoot apex.

The Formation of Leaves

Klein (1884) described the formation of leaves in *P. aquilinum*. The leaf primordia are formed singly at the apical meristem, and their development is very slow. According to Klein the leaf takes four years from its inception to develop into a mature leaf. Hofmeister had stated earlier (1857) that this period was three years, and according to Webster and Steeves (1958) three growing seasons are usually required for complete leaf development in the var. *latiusculum* which they investigated.

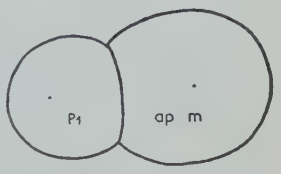

Fig. 8. Plan of apical meristem and youngest leaf (P_1, in late plastochrone) of a long shoot apex. (×60)

Long shoots. Because of the long plastochrone and slow development, the observation of leaf formation and development is a difficult matter. The apical meristem comprises the apical cell and segments formed by it on either side (Plate, 1). The apical meristem extends on one side by greater growth, and one of the cells here develops into the leaf apical cell (Fig. 8; Plate, 6 and 7). The latter is two-sided like the shoot apical cell, but a leaf primordium can be distinguished from the apical meristem, particularly in horizontal longitudinal sections, by its stronger curvature, especially of the outer walls of its apical cell (Plate, 6). The angle of divergence between successive leaf primordia at the vertex of the apical meristem is about 150°.

An interesting feature of the further development of the leaf primordium is that the curvature of the tip of the older leaf primordium is not towards the apical meristem, as in *Dryopteris aristata*, *Polypodium vulgare*, and many other ferns, but towards the rear of the parent shoot, in a direction between that of the apical meristem and the abaxial bud (Figs. 1 and 2). Leaves borne on both long and short shoots show this relationship.

Short shoots. The formation of leaves at short shoot apices is essentially similar. Owing to the slow growth and elongation of short shoots, more than one leaf primordium is often found at a shoot apex (Plate, 8).

The Formation of Buds

Long shoots. Fig. 9 and Plate, 9 show long shoot apices with older P_1 leaf primordia, which have been slightly separated from the apical meristem by the growth of the latter. A bulge can be seen low on the abaxial side of the leaf primordium, which is the abaxial bud. Plate, 10 is a transverse section of a long shoot apex, showing the leaf and bud. The adaxial bud cannot be

distinguished at this stage, but it appears to become organized later from meristematic cells at the adaxial base of the leaf primordium (Plate, 11).

With the further separation of the leaf primordium from the elongating distal end of the shoot, an up-growth of tissue takes place in the axis beneath the leaf primordium. As a result of this, and of the continued growth of the leaf primordium, the two buds become separated from the latter, and stand on either side of it at the top of a hump on the axis. Plate, 12 (i), shows the appearance of one of these humps. When the apex of such a hump is bared of scales, it appears as in Fig. 10. The leaf primordium is seen, flanked on the abaxial and adaxial side by buds, the abaxial bud being the larger of the two. This bud proceeds to grow strongly, carrying the leaf primordium with it, which is thereby turned to an adaxial position, appearing to arise from a lateral shoot (Fig. 2; Plate, 12 iii). The adaxial bud remains undeveloped, usually developing further only following injury to the abaxial one.

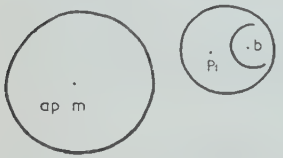

FIG. 9. Plan of long shoot apex, showing apical meristem, leaf primordium P_1 and abaxial bud, *b*. (×60)

Short shoots. The formation and development of buds in short shoot apices is similar, with the difference that only abaxial buds are formed at the base of the leaf primordia. A further difference is that the elevation of the leaf primordium on a lateral hump is not so marked as in long shoots, and the growth of the bud is much slower. The bud, in fact, ceases growth at an early stage, and remains at the base of the leaf as a small hump (Fig. 1).

The origin of buds. Direct examination of shoot apices and examination of sections show that buds in *P. aquilinum* have their origin at the apical meristem. When a leaf primordium forms, the apical meristem broadens in a dorsolateral direction, the leaf primordium arising on the resulting broad upgrowth (Fig. 8; Plate, 6). It is from meristematic tissue at the edge of the apical meristem, abaxial to the leaf primordium, and at its base, that the abaxial bud originates. Although the abaxial bud is not recognizable as such while the leaf primordium is at the apical meristem, its origin from meristematic tissue at the base of the growing leaf primordium can be followed. The temporary absence of an adaxial bud until the leaf primordium is some distance away from the apical meristem may be due to an inhibitory effect of this region: this should be amenable to experimental verification. Experimental investigation may also throw light on the absence of an adaxial bud on leaves of short shoots. This may also be a result of inhibition by the apical meristem: the leaf primordia of the slow-growing short shoots remain a long time in the proximity of the apical meristem, which probably prevents the formation of a bud from meristematic tissue at its adaxial face.

With regard to the vascular structure of the part of the leaf base between the abaxial bud and the main axis, Mettenius (1863) observed that if the bud at the leaf base develops late, the vascular bundles of the bud and leaf are completely separate, while if the development of the bud precedes that of the

leaf, then the vascular structure of this region resembles that of the main axis, the bundles of the leaf and bud being fused. The writer's observations are in agreement; the explanation for the difference in vascular structure appears to be that, where (in long shoots) the bud develops strongly at an early stage, the prevascular tissue proximal to it and also that of the leaf base are induced to differentiate in a manner resembling that of stems, while in short shoots, where the bud and leaf initially develop about equally, the prevascular tissue proximal to them differentiates more or less independently, each in its characteristic manner.

FIG. 10. Plan of apex of a lateral hump similar to that in Plate, 12 (i), showing leaf primordium and the two buds. (×35)

DISCUSSION

The observations on the construction of the shoot system and its morphogenesis in *P. aquilinum* present a number of problems for further experimental investigation. One of these is the cessation of growth of the radial shoot apex of the sporeling with the formation of two dorsiventral lateral shoots. Direct observation of shoot apices showed that these lateral shoots arise as buds on the margin of the apical meristem. But why the original apical cell group ceases growth and why the new buds give rise to dorsiventral shoots is not known.

A condition similar to *P. aquilinum* has been reported by R. and C. Wetter (1954) in *Stenochlaena palustris* where they state that the radial axis of the sporeling branches into two shanks, these developing as dorsiventral shoots. A somewhat similar condition appears to exist in some angiosperms. According to Clark (1904), species of *Cyanotis* have radial main axes which produce dorsiventral lateral branches, and Troll (1937) has cited other similar instances. Also M. and R. Snow (1935) found that when the apex of the decussate shoot of *Epilobium hirsutum* was split in two, the halves grew out into shoots which had radially arranged leaves.

The tip of a leaf primordium is normally bent towards the parent apical meristem, but it is known in some other ferns that where the apical meristem is weakened by injury the direction of curvature may alter. Thus, Cutter (1954) and Wardlaw (1955) have shown from experimental investigations on the development of leaf primordia in *Dryopteris aristata* that the symmetry and orientation of primordia are controlled by the organization and the physiological activity of its environment at the shoot apex, and Wardlaw and Cutter (1955) found that when the apical cell group of *D. aristata* was lightly punctured, some of the subsequently formed leaf primordia were orientated

towards new buds which formed in their vicinity or actually curved away from the apical meristem. In *P. aquilinum* the orientation of the young leaf primordium may be influenced by its position in relation to the distribution of growth at the shoot apex, e.g. the greater extension of the ventral region, in addition to any possible regulation by the bud in its vicinity. Eventually the young leaf is neither curved towards the parent apical meristem, nor towards the abaxial bud, which is supposed by some workers to be the parent meristem, but in an intermediate direction, between the two.

The observations presented here regarding the formation of buds show that, as in other ferns, there is here a 'unity of meristematic tissue throughout the plant' (Wardlaw, 1943) with buds developing from meristematic tissue referable in origin to the apical meristem. Buds in *P. aquilinum* are thus neither adventitious nor the result of dichotomy of the main axis.

Leaves arise from the apical meristem as in other ferns, with no difference in this respect between the two types of shoot. Leaves and abaxial buds arise almost concurrently from cells of the apical meristem, the leaves being formed at the apical meristem, and not by the bud. The position of the buds in relation to the leaves is such, however, that during their further development the buds remain in close association with the leaf bases. Later, owing to the distribution of growth at the shoot apex, changes occur in the spatial relationships of the buds and leaves, and finally in long shoots the leaf appears to have been produced by the bud, while in short shoots the bud is carried up on the leaf base. Wardlaw (1943) has pointed out that such displacement of buds in relation to leaves is determined by the specific distribution of growth in the shoot and leaf base, and Kundu and Rao (1957) have reported the same occurrence in some angiosperms. The present observations are in agreement with Mettenius' (1860) views on the development of buds in *P. aquilinum* in relation to leaves.

The difference in form and structure of long and short shoots is at least partly referable to differences in their growth-rates. The internodes in long shoots elongate more quickly and to a greater extent than in short shoots, and long shoot apices and buds borne on them also have a faster rate of growth. The two types of shoot are thus similar to long and short shoots where they occur in other plants, and in this connexion the recent work on long and short shoots of *Ginkgo biloba* by Gunckel *et al.* (1946, 1949) is of great interest (*see also* Wetmore, 1956). Gunckel and his co-workers found that 'there is no fundamental difference in the cellular organization of the apical meristems in long and short shoots', and that the development of the two types of shoot was correlated with the quantity of available auxin. Webster and Steeves (1958) expect to investigate the shoot system of *P. aquilinum* along similar lines, and a report of their findings is awaited with interest.

A further point in connexion with the presence of the two types of shoot is that in some soil types they grow at different levels in the soil, moving from one level to another with a change in the type of shoot. Much experimental and physiological investigation of the shoot apex in this fern is necessary

before solutions can be found to the many problems presented by its morphology.

Summary

1. The rhizome of *P. aquilinum* is dorsiventral, with leaves in two dorsal rows and roots all round. The shoot system of the adult plant is made up of two main types of shoot—long shoots and short shoots. Long shoots have longer internodes, and their growth and extension is more rapid than in short shoots.

2. The sporeling has an erect axis, with a three-sided apical cell. During the development of the sporeling the apical meristem ceases growth, and two lateral buds appearing at its edges grow out into horizontal dorsiventral axes with two-sided apical cells.

3. Leaves have their inception at the apical meristem. Leaf inception on long and short shoots is similar. During their further development the leaf primordia become curved towards the rear of the parent shoot.

4. Leaves produced on both long and short shoots commonly have a bud at the base, on the abaxial face, produced from meristematic cells separating from the edge of the apical meristem during the inception of leaves. In long shoots this bud grows strongly from an early stage, with the result that the leaf is carried away from the main axis, and appears to be produced by the bud. Leaves on long shoots bear, in addition, an adaxial bud. Differences between long and short shoots are attributed mainly to the quicker growth and internodal elongation of the former.

Acknowledgements

The writer wishes to thank Professor C. W. Wardlaw for his helpful guidance and encouragement throughout the investigation, and Dr. E. G. Cutter for many valuable suggestions. His thanks are also due to Mr. E. Ashby and Mr. G. Barker for the photographic illustrations.

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EXPLANATION OF PLATE

1. Transverse section of long shoot apex, passing through the apical meristem (*a*) and the youngest leaf (P_1). Note two-sided apical cells of both shoot and leaf. (×60)

2. Transverse section of shoot apex of very young sporeling. Note three-sided apical cell (*ac*). *l*, leaf. (×120)

3. Median longitudinal section of shoot apex of very young sporeling. (×120)

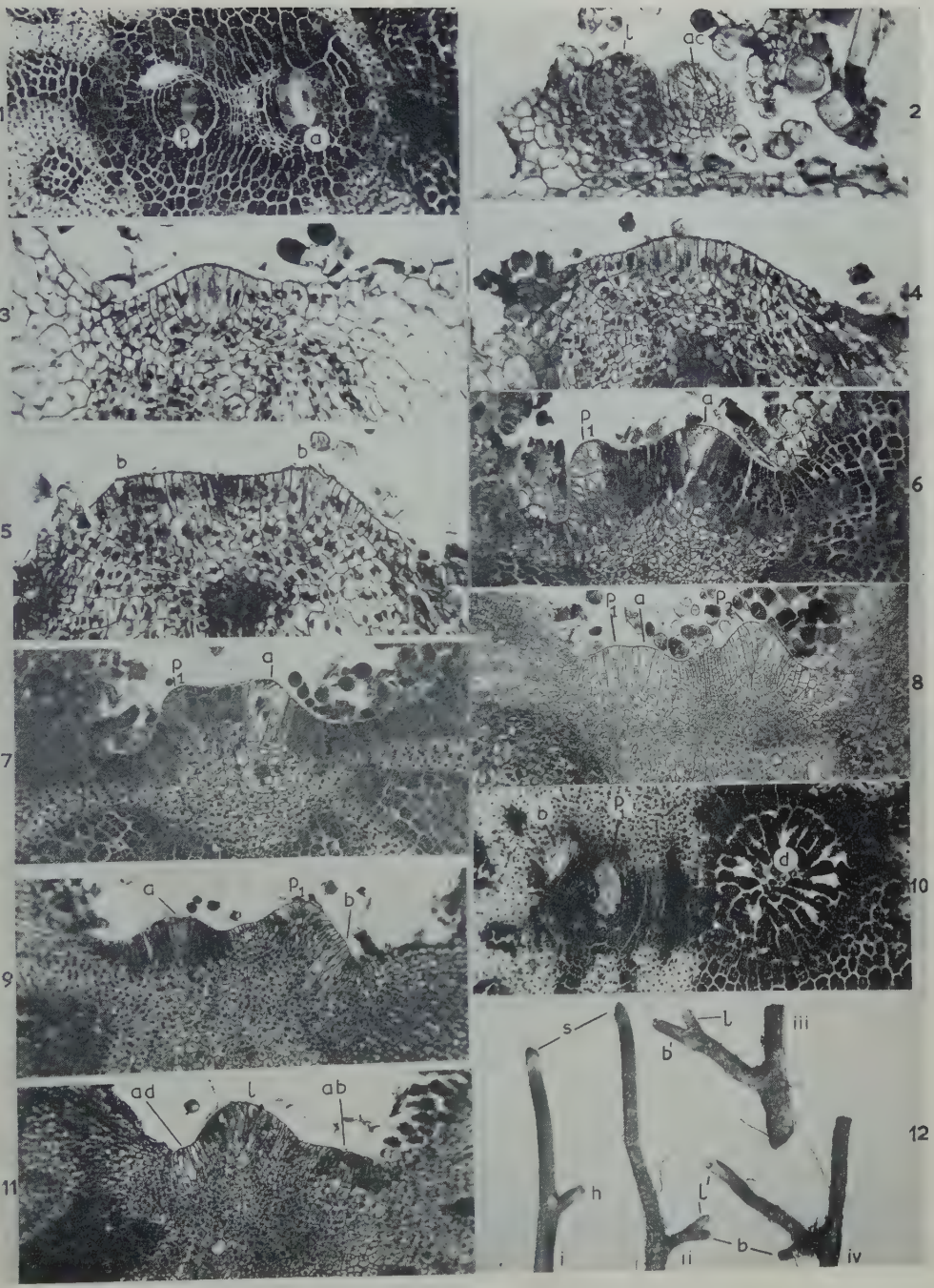
4. Median longitudinal section of shoot apex of older sporeling, showing broadening of apical meristem. (×120)

5. Longitudinal section of shoot apex of older sporeling, showing two buds, *b*, *b*. (×120)

6. Median longitudinal section through apical meristem (*a*) and P_1 of long shoot apex illustrated in Text-figure 8. (×60)

7. Median longitudinal section through apical meristem (*a*) and P_1 of a long shoot apex similar to that illustrated in Text-figure 8. Note meristematic cells at abaxial base of leaf primordium. (×60)

8. Horizontal longitudinal section of short shoot apex, showing apical meristem (*a*) and two leaf primordia P_1 and P_2. The apical meristem is cut tangentially. (×60)



M. D. DASANAYAKE

9. Longitudinal section of long shoot apex illustrated in Text-figure 9. The section passes tangentially through the apical meristem (*a*) and P_1. *b*, abaxial bud. (×60)

10. Transverse section of long shoot apex, showing abaxial bud (*b*), leaf primordium P_1 and apical depression (*d*) occupied by the apical meristem (covered with hairs). No adaxial bud can be recognized. (×60)

11. Longitudinal section of long shoot apex, showing leaf primordium (*l*) with adaxial (*ad*) and abaxial (*ab*) buds. The abaxial bud is cut tangentially. (×60)

12. Production of leaves and buds by long shoots. (i) lateral hump (*h*) close to distal end of shoot(*s*); (ii) young leaf (*l*) and abaxial bud (*b*) growing out independently; (iii) abaxial bud (*b*) growing out vigorously, the leaf (*l*) pushed to a lateral position; adaxial bud not seen; (iv) growing leaf (*l*), abaxial bud (*b*) relatively less developed. *s*, shoot apex; *b*, bud; *l*, leaf. (×$\frac{2}{3}$)

The Breakdown of Sucrose During Translocation

BY

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With two Plates and one Figure in the Text

Abstract

When a vine petiole is carrying labelled sucrose away from the lamina, the quantity of labelled carbon dioxide lost from the petiole bears a constant relation to the quantity of labelled sucrose inside the petiole. Sucrose is virtually the only labelled sugar in the petiole, and the labelled sucrose is confined to part of the phloem. Calculations based on these measurements and some assumptions suggest that the rate of breakdown of the translocated sucrose is about 0·5 mg. per c.c. of phloem per hour. The bearings of these findings on the problem of energy supply to translocation are discussed.

Introduction

THE supply of energy to the process by which carbohydrates are transported in plants is almost completely mysterious. All authorities agree that the process uses metabolic energy and that this is probably derived from respiration, but about the source, magnitude, and means of application of this energy nothing is known. So far as can be discovered from the literature, considerations of the possible magnitude of the energy required to perform the measured movements have been few and none have been made in the light of recent work. Mason, Maskell, and Phillis (1936), in an introduction to one of their series of papers on translocation in cotton, argue against the streaming hypothesis on the basis of the large expenditure of energy which would be necessary to maintain the observed rates. In a footnote on page 29 of that paper they detail a calculation of the energy needed to maintain the estimated gradient of 180 atmospheres pressure per metre of sieve-tube path, and arrive at a figure of 1,060 cals. per c.c. of sieve-tube track per 24 hours, which, they point out, would require 'the respiration of at least 0·25 g. of sucrose per 1 c.c. of sieve-tube sap per day, i.e. a 25 per cent. solution of sucrose in the sieve-tube sap would be completely exhausted in one day'. In the same paper they say that the rate of application of energy necessary for their proposed mechanism of activated diffusion cannot be calculated. An error has been revealed in these calculations by Palmquist (1938). The rate of streaming calculated by Mason *et al.* is based on the wrong gradient, that across a single sieve-plate instead of that along a whole centimetre as would be consistent with the other units, giving a streaming rate twenty times larger

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than is justified by the measurements. This affects the driving pressure necessary as well as the velocity, and the recalculated value of the energy required is 26 cals. or 0·004 g. of sucrose per c.c. of sieve-tube per day.

Evidence bearing indirectly on the problem of the energy supply to translocation has been accumulating in the last few years in the form of measurements of the respiration of isolated phloem and vascular bundles. (For a review of this literature see Esau *et al.* (1957).) Although the very high values for carbon-dioxide output of isolated phloem found by Russian workers (up to 5,000 μl./g. fw./hr.) are unsubstantiated, the respiration rate of vascular bundles seems higher than that of the ground parenchyma in most systems studied; values of 220 μl. CO_2/g. fw./hr. being commonly found for isolated bundles in sugar buffers, compared to about 100 μl./g. fw./hr. for ground parenchyma. The substrate was presumably the sugar in the buffer and it has been inferred, not proved, that this high respiratory activity is related to the translocation of organic substances in the vascular tissues. The substrate for phloem respiration in an intact organ must be the carbohydrates of the surrounding tissue or the translocated sucrose itself, and there is at present no evidence to show that the high rate of respiration is maintained by the phloem in the plant or that the energy derived from it is directly applied to moving sucrose in the phloem.

The related problem of the integrity of the transported carbohydrate has also apparently escaped the attention of workers in this field. It seems to be of fundamental importance to know whether the translocated sucrose is subject to respiratory breakdown *en route*, and if so, what proportion of the translocated sucrose suffers such breakdown. The respiratory energy for the movement must be derived from stored carbohydrate along or at the ends of the path, or from the translocated sucrose itself, or both, and the tools are already in our hands by which measurements of these processes can be attempted. If the energy source is labelled radioactively, radioactive carbon dioxide will give an estimate of the rate of breakdown of the source, and if this source is largely or wholly the translocated sucrose, a fair estimate of the minimum energy consumed can be made from the output of labelled carbon dioxide by an organ which is translocating labelled sucrose. Willenbrink (1957) surrounded vascular bundles of *Pelargonium* with gas chambers and applied various atmospheres to the transporting tissue, and also detected the passage of labelled molecules through the bundles with a sensitive device. He did not seek to record the presence of labelled carbon dioxide in the chambers derived from labelled sugars in the translocation stream. This paper is an account of an experiment designed first to detect and, having detected, to extract meaningful data from, the release of a portion of the translocated sucrose as carbon dioxide.

Methods

The experiments were performed with the grapevine *Vitis vinifera* L., variety 'Red Cornichon', on current-season's canes in the autumn. The vine

was selected because it has a well-developed phloem lying close to the surface, no lenticels, and long, uniform petioles and internodes which may be enclosed in a simple chamber. A long cane was cut from the plant and its end cut under water. The cane was supplied with water and set up by a window where it was illuminated by diffuse skylight for 10–11 hours each day. No illumination was supplied at night, nor was temperature control attempted. Canes preserved in this way showed no signs of deterioration in a week. The part of the plant to be investigated was enclosed in a gas-tight, opaque, plastic chamber 76 mm. long, sealed at the ends with cocoa-butter as shown in Plate 1, and a gas stream was passed through the chamber by connecting it to an evacuated flask through a screw-valve. Laboratory air was freed from CO_2 in a soda-lime tower, bubbled through lime-water, passed through the respiratory chamber and into a Pettenkofer tube containing 15·0 ml. of 0·2 *N* NaOH. Leaks in the chambers were manifested by the cessation of bubbles in the lime-water bottle. After a period of collection of carbon dioxide, 7 hours during the day or 17 at night, the carbonate was precipitated with 30 ml. of 0·05 M $BaCl_2$. The remaining sodium hydroxide was titrated against standard hydrochloric acid using phenol-phthalein indicator, and the total carbonate produced was calculated. The precipitated carbonate was filtered, washed, and counted with an end-window Geiger tube (EW_3H), giving a measure of the output of labelled carbon dioxide from the enclosed organ.

Radioactive sucrose was introduced into the plant by allowing a leaf to make its own sucrose from $^{14}CO_2$ in daylight. Preliminary trials showed that sucrose introduced in this way moved much more extensively than when introduced as labelled sucrose in alcoholic detergent solution on the leaf surface. This has been our general experience with many different leaves, and presumably indicates that the sucrose formed in photosynthesis is in some way more conveniently situated for entry into the translocation system than foreign sucrose from outside the cells. Labelled CO_2 was liberated from 1 mg. of $Ba^{14}CO_3$ (5 μc) by 80 per cent. lactic acid in a small bottle sealed to the lower leaf surface with petroleum jelly as may be seen in Plate 1. All labelled carbon dioxide had disappeared from the bottle after about 2 hours from the adding of lactic acid, so no care was taken to see that bottles remained attached to the leaves after this time.

Extractions of the sugars inside the organs enclosed by the chambers were made by blending in hot 70 per cent. ethanol. The filtrate was stored at −20° C. if storage was necessary, and an aliquot of the filtered ethanolic extract was evaporated directly on to acid-washed Whatman No. 3 mm. paper and chromatographed in ethyl acetate/pyridine/water (80 : 20 : 10) for about 20 hours. Test strips indicated the positions of specific sugar spots, and the distribution of radioactivity on the chromatogram was determined by an automatic scanner yielding a trace proportional to the quantity of radioactivity along the strip. This estimate of radioactivity could not be compared directly with that of the barium carbonate determined in the end-window counter, so the various sugar spots were eluted and burned in a closed system with a Van Slyke–Folch

oxidation mixture, the carbon dioxide precipitated as barium carbonate, and this counted in the same system with the same geometry as the samples from the respiratory gas stream. Additional sugar was added to the eluted spot before combustion to provide a convenient body of precipitate for filtering. This diluted the counts obtained, but still permitted the comparison of the two activities.

Estimates of the quantity of sugar in eluted spots were made by a modification of the method of Wager (1954). Sucrose was hydrolysed before estimation in hot dilute hydrochloric acid.

The fraction of the phloem carrying labelled assimilate. To complete the data on transport through the tissues enclosed in the chamber, an estimate was attempted of the fraction of phloem carrying labelled assimilate in the following way. At the time when the respiration chamber was dismantled and the organ within it taken for extraction, the adjacent pieces of the organ outside the chamber at each end were sectioned with a razor blade and immediately frozen on dry ice. The sections and dry ice were placed with calcium chloride in a vacuum desiccator at −20° C. and the desiccator evacuated for 2 days, after which it was warmed to room temperature and the vacuum very slowly released. The sections were exposed to X-ray film for long enough to give a suitable image (2–10 days), and both the sections and radiographs were photographed. The areas were measured in the enlarged photographs of (1) the cross-section of the organ, (2) the cross-section of the phloem, and (3) the cross-section of the phloem which produced a radiograph. A typical pair of such photographs is shown in Plate 2 and corresponds to the petiole number 3 in Table 2.

Results and Discussion

Time Course of Output of $^{14}CO_2$

The total output of $^{14}CO_2$ from the enclosed organ during a period is clearly a measure of the amount of breakdown of labelled sugar inside the organ during the period. This output of $^{14}CO_2$ is measured by the total counts from the barium carbonate derived from the gas stream, and is proportional to the product of the specific activity of the precipitated carbonate and the mass of the precipitate, and hence also to the product:

$$\text{counts at infinite thickness} \times \text{precipitate mass.}$$

A leak in the gas system which admits aerial CO_2, or the presence of CO_2 derived from respiration of substances other than sucrose, do not invalidate this measure, since the counts are reduced in the same proportion that the precipitate mass is increased. If now we wish to compare the rate of breakdown of labelled sugar in the organ during this period with that during another period of different length and during which the temperature was different, we might try to express both breakdowns in terms of unit time and a standard temperature. On the other hand, both these variables, time and temperature, will presumably affect the total respiration of the tissue (total CO_2 output) in

exactly the same way as they affect the breakdown of labelled sugar, and a satisfactory measure for comparison should be:

$$\frac{\text{sugar* breakdown}}{CO_2 \text{ output}} = \frac{\text{sugar* breakdown}}{\text{precipitate mass}}$$

$$= \frac{\text{counts at infinite thickness} \times \text{precipitate mass}}{\text{precipitate mass}}$$

$$= \text{counts at infinite thickness.}$$

Notice that it is purely fortuitous that the precipitate mass appears in both top and bottom lines of this fraction, and also that while extraneous CO_2, from a leak, &c., does not alter the measure of the amount of sugar breakdown, it does alter the measure of temperature and time by the precipitate mass, for the leak will not vary much with temperature. It is of the greatest importance, however, that we may expect the single measurement of the counts of the precipitate at infinite thickness to give us a satisfactory measure of the rate of sucrose-^{14}C breakdown corrected for temperature, for comparison from one sampling period to another. This measure is only valid for comparisons on the same organ at different times, not between different organs.

This measure was used to follow the time-course of breakdown of labelled sugar in a petiole carrying the products of photosynthesis away from a lamina treated with $^{14}CO_2$. The application, sampling, and counting were carried out as described under 'Methods' for a system like that in Plate 1, and the counts at infinite thickness for the successive samples of respiratory carbonate are plotted against the time in hours from the application as Fig. 1. The curve is not always so smooth; there can be a stepwise variation between day and night, and if there is a leak of $^{14}CO_2$ out of the application bottle and into the nearby chamber, the first reading can be abnormally high, but broadly the course is always of the form shown; a rapid rise to a maximum rate after something less than 20 hours, and a steady high level maintained for 2 to 3 days, followed by a slow fall. This can be taken to indicate that the reservoir of labelled substances formed in the leaf during the short exposure of a few hours to $^{14}CO_2$ is sufficient to saturate the transporting system with labelled sugar for up to 3 days and that during the 2nd and 3rd day a steady flow of labelled substances through the petiole is accompanied by a steady respiratory loss of these substances as $^{14}CO_2$. We may note in passing that this implies the transport system is always normally supplied with more leaf-sugar than it can move, but whether the rate-limiting process is diffusion to the phloem or translocation in the phloem is uncertain. The time 30 hours after application, when the rate of output of $^{14}CO_2$ was steady, was chosen, on the basis of such curves as Fig. 1, as that most suitable for making the comparison between the labelled carbon dioxide coming from the petiole and the labelled sugars within the petiole. At this time the apparatus was dismantled, the petioles cut from the chambers, weighed, and extracted as described above.

The Labelled Sugars of the Tissues

Spraying of chromatograms of the ethanolic extract of the tissues within the chamber revealed only sucrose, glucose, and fructose; and scanning the chromatograms for radioactivity revealed only one radioactive spot, the

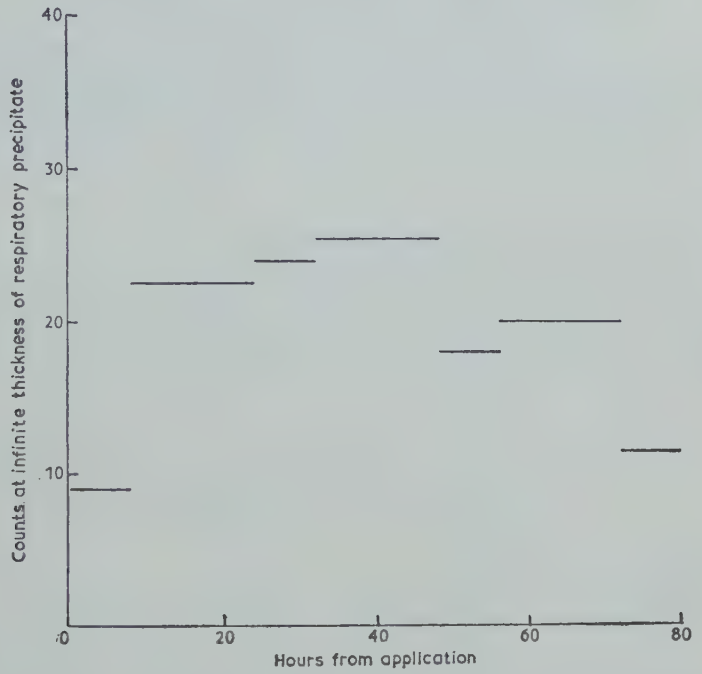

FIG. 1. Plot of the counts at infinite thickness of the carbonate precipitate from the respiratory gas stream of a petiole against the time from the application of $^{14}CO_2$ to the lamina. This gives a measure of the rate of sucrose-^{14}C breakdown within the petiole during the different sampling periods (see text).

TABLE 1

Relative Labelling of Sugars in a Petiole

(*Data for petiole 8 of Table 2. Mass of tissue extracted 0·53 g.*)

Sugar	Mass of sugar mg.	Total radioactivity c.p.m.
Sucrose	4·60	25,500
Glucose	5·25	1,690
Fructose	0·53	2,045

sucrose spot. As this method of detection of radioactivity is less sensitive than the end-window counting of barium carbonate, a further check was made by elution and combustion of spots from the chromatogram of one of the most heavily labelled extracts (petiole 8, Table 2). The relative radioactivities were as shown in Table 1. It will be seen that nearly 90 per cent. of the radioactivity of the extract is contained in the sucrose. Further, radiography of

organs through which sucrose-^{14}C had passed showed no detectable labelled compounds remaining after treatment with alcohol; so, for our purposes, we may assume that the only source of $^{14}CO_2$ within the organ was sucrose-^{14}C.

The Proportion of the Labelled Sucrose within the Tissue that is Broken Down

The two sets of barium carbonate counts, that from the gas stream outside the organ and that from the extracted and burned sucrose within, yield, when multiplied by their masses, measures of the total radioactivity from the two

TABLE 2

Proportion of Instantaneous Sucrose Content Lost in Seven Hours Preceding Extraction

Sample	Total radioactivity of sucrose (c.p.m.)	Total radioactivity of respired CO_2 (c.p.m.)	CO_2/sucrose ($Q \times 100$)
Petiole 1	1,288	66	5·1
,, 2	6,250	310	5·0
,, 3	4,275	169	4·0
,, 4	6,100	326	5·4
,, 5	3,730	221	5·9
,, 6	10,990	328	3·0
,, 7	25,780	855	3·3
,, 8	25,500	2,780	10·5
Stem 1	8,155	238	2·9

sources, but these are on different bases. That from the gas stream is proportional to an amount of sugar breakdown in the preceding sampling period (the 7 hours of the 2nd day in these experiments) and that from the organ is proportional to the instantaneous sucrose content of the petiole. Curves like that in Fig. 1 encourage us to believe that the instantaneous sucrose content is fairly steady and we may compare the two measures if we remember what they represent. Table 2 lists the results of eight experiments of the type just described. The lamina of a leaf is fed $^{14}CO_2$; the petiole-CO_2 is sampled for that day, the following night and day; and the extractions are made at the end of the 2nd day. One experiment is also included in Table 2 on stem tissue, in which part of a stem internode above treated leaves was enclosed in a chamber and sampled in the same way, and extracted at the same time as the petioles. The second and third columns contain the measurements of radioactivity inside and out. The units are arbitrary—counts per minute—but all comparable with one another, having been corrected for background, drift, and sample thickness. Each radioactivity entry represents the product of counts at infinite thickness and precipitate mass. The fourth column lists the ratio of the radioactivity from respired CO_2 to that inside the organ (a quantity we shall call Q) multiplied by 100. That is, it is the percentage of the instantaneous sucrose content which was lost by respiration during the preceding 7 hours.

It is clear that despite a wide range in the richness of labelling of the sucrose within the organ and the carbon dioxide without, there is a very remarkable constancy in the proportion that the one bears to the other. We shall consider what this proportion means in terms of quantity of sucrose respired.

The Quantity of Sucrose Broken Down

The simple expression:

$$\frac{\text{sugar breakdown}}{\text{sugar content}} = \frac{(\text{counts} \times \text{ppt. mass}) \text{ from gas stream}}{(\text{counts} \times \text{ppt. mass}) \text{ from combustion of extracted sugar spot}} = Q$$

would allow us to calculate the quantity of sucrose broken down if we knew the mass of sucrose at the site of breakdown, while all we have measured is the total mass of sucrose in the whole organ inside the chamber. The site of breakdown may be identified as the phloem from radiographs of the organ like that of petiole 3 in Plate 2, and the problem resolves itself into estimating

TABLE 3

Sucrose Breakdown Corresponding to Various Estimates of the Sucrose Content of the Phloem

Sample	Sucrose content mg.	$Q \times 100$	Phloem volume as fraction of whole	Maximum breakdown (μg./7 hr.)	Minimum breakdown: Sucrose at site (μg.)	Minimum breakdown: Loss (μg./7 hr.)	Probable breakdown: Sucrose at site (μg.)	Probable breakdown: Loss (μg./7 hr.)
Petiole 1	5·25	5·1	0·055	268	289	15	1,794	91
,, 2	4·45	5·0	0·07	222	312	16	2,048	102
,, 3	4·40	4·0	0·07	175	308	12	2,582	104
,, 4	4·35	5·4	0·07	234	304	16	2,736	148
,, 5	5·35	5·9	0·07	316	374	22	3,346	197
,, 6	7·00	3·0	0·05	210	350	10	4,717	141
,, 7	5·80	3·3	0·06	192	348	11	3,538	117
,, 8	4·60	10·5	0·06	482	276	29	2,635	276
Stem 1	27·80	2·9	0·08	805	2,220	64	20,612	600

the mass of sucrose contained in the phloem. We might base our estimate on the results of other workers who have tried to estimate the concentration of the translocated sucrose solution from data on phloem exudates and by indirect means, but this is such a vexed question that it is preferred to attempt an independent estimate.

The sucrose content of each of the experimental organs is listed in Table 3, but the mass of sucrose in the phloem would be this quantity only if there were none in any other tissue. The product of this mass and the ratio Q for each organ gives the *maximum* breakdown of sucrose in the phloem in the 7 hours preceding extraction, and the quantities calculated thus are listed in Table 3. It might be thought that a further correction should be made because only a fraction of the phloem is carrying sucrose-^{14}C, but a moment's thought will make it clear that the relation still holds, for the counts from the gas stream will vary in the same proportion as the counts from the sucrose in

the organ if different proportions of the phloem are labelled. This reasoning is confirmed by the constancy of the ratio Q from one organ to another in which different fractions of phloem are carrying sucrose-^{14}C.

At the opposite end of the scale, if the sucrose of the organ were spread evenly throughout each of its tissues, the mass contained in the phloem would be the product of the sucrose content and the fraction of the whole volume occupied by the phloem. The corresponding *minimum* sucrose breakdowns, based on Q times this sucrose mass, are also listed in Table 3. The more extreme case in which there is a smaller concentration of sucrose in the phloem than in the surrounding tissue does not seem worth considering. The actual sucrose breakdown must lie between the estimated *maximum* and *minimum*, and let us see if we can find reasons for placing it near one end of the range.

Rough dissections were made of petioles to separate them into three regions: the central pith, the region of the vascular bundles, and the cortex outside the vascular bundles. This last was almost impossible to obtain free from phloem since the bundles lie so close to the surface. Each region was extracted with hot 80 per cent. ethanol; the sugars were separated by chromatography; and the sucrose, glucose, and fructose spots were eluted and determined. Hence the sugar concentrations of the three regions in mg. per gram fresh-weight were calculated, and the sucrose values found to be: pith, 3; cortex, 5; vascular region, 6. The pith region contains least sucrose, and the vascular region at least twice as much. The cortex is intermediate, but in view of the above-mentioned difficulty of separation, probably contains extra sucrose from the richer vascular region, just as the vascular region no doubt contains a small volume of concentrated sucrose diluted by a larger volume with less sucrose. As a basis for calculation, let us assume that all the tissue which is not phloem contains a uniform concentration of 3 mg./g. fw. of sucrose; then we may calculate for each organ the mass contained in the phloem, knowing the total content and the fraction of the whole volume which is occupied by phloem. The results so obtained (with the corresponding breakdown figures) constitute the third section of Table 3, and will be taken as a likely estimate of the breakdown of translocated sucrose in the phloem in the 7 hours preceding extraction.

The interest of these breakdown figures lies in their relation to two things: considered as respiratory breakdown, to the respiration of the whole organ; and considered as loss of translocated carbohydrate, to the total sucrose which has passed through each organ during the sampling period. Let us consider them in that order.

Contribution of Translocated Sucrose to the Respiration of the Organ

During the 7-hour sampling period prior to extraction, the total carbonate from each organ was measured as part of the routine ^{14}C-assay and as long as there was no leak in the chamber admitting aerial CO_2, this should represent the respiratory CO_2 output of the organ. We may compare this with the CO_2

output of the phloem calculated from the probable amount of sucrose broken down there. The two respiratory quantities are compared in Table 4 on the basis of CO_2 output in μl./g. fw./hr. after the reduction of the sucrose breakdown figures from Table 3 to suitable units. For this reduction it is necessary to assume a density for the phloem since the photographic measurements yielded an estimate of its volume not its mass, and a density of 1 has been used for want of a better figure. Although the densities of the whole organs estimated in Table 4 from the known mass, length, and cross-sectional areas are all 1 or less, the denser parts are probably close to 1 g./c.c.

TABLE 4

Contribution of Phloem to Respiration of Organ

				Phloem respiration		Organ respiration		
Sample	Mass g.	Phloem volume c.mm.	Estimated density g./c.c.	Probable loss μg./c.mm./7 hr. sucrose	Equivalent CO_2 loss μl./g. fw./hr.	Total $BaCO_3$ mg./7 hr.	CO_2 μl./g. fw./hr.	Ratio of total respiration phloem/organ
Petiole 1	0·72	68	0·6	1·34	100	22	499	0·02
,, 2	0·63	66	0·7	1·55	120	33	854	0·02
,, 3	0·50	44	0·9	1·76	130	20	654	0·03
,, 4	0·60	42	1	3·52	260	17	462	0·04
,, 5	0·72	52	0·8	3·79	280	19	430	0·05
,, 6	0·56	39	0·6	3·61	270	18	390	0·04
,, 7	0·53	46	0·6	2·54	190	19	574	0·03
,, 8	0·53	45	0·8	6·14	460	21	645	0·07
Stem 1	2·43	204	1	2·94	220	29	195	0·08

The estimated rates of evolution of carbon dioxide derived from translocated sucrose in the phloem are around 200 μl./g. fw./hr., a figure comparable with that found by other workers for isolated phloem in sugar buffers. The view of phloem as a tissue of high metabolic activity is scarcely supported by these measurements, but we have measured here only that part of the phloem respiration which uses the translocated sucrose as substrate and there may be a large contribution from other substrates. More surprising are the consistently high figures for respiration of the whole petioles, while the stem is giving off carbon dioxide at a more orthodox rate. Some of the very high values like that for petiole 2 may be due to leaks in chambers admitting aerial CO_2, but this cannot be true of all the samples or at all periods other than this 7-hour one when similar rates were measured. The stem figures incline us to confidence in the sealing of at least some of the chambers, and yet the lowest rate for a petiole is well above what is normally considered a moderate rate for an intact plant organ. The contribution of the translocated sucrose in the phloem to the whole respiration of the organ is small, and less even than the proportion of the volume of tissue occupied by phloem, as will be seen by comparing the ratios of respiration in the last column of Table 4 with the volume ratios in Table 3.

Proportion of the Translocated Sucrose Lost in Transit

The quantity of translocated sucrose which has passed through the experimental organs in these experiments has not been measured, nor is it readily

accessible to direct measurement by any means at our disposal simultaneously with the measurements recorded above. We may therefore either estimate this quantity from the results of other workers on other systems, or use the above data to get a rough indirect estimate. Direct measurements of the mass transfer of sucrose in phloem are few and have usually been made on systems selected to demonstrate the highest rates attainable rather than the rates normally realized in the parts of growing shoots. All those that give results in terms of velocities are useless to us for the present purpose, and only those from which we can extract a mass-transfer rate are helpful. These are: the measurements of Mason and Lewin (1926) on yams; those of Clements (1940) on the Sausage Tree; those of Crafts and Lorenz (1944) on squash fruits; and the data of Colwell on squashes quoted by Crafts and Lorenz. The mass-transfer rates that may be extracted from their data are shown in Table 5.

TABLE 5

Mass Transfer of Dry Weight Through the Phloem, Calculated from Published Data

		Mass Transfer (g. dry wt. per cm.^{2} of phloem per hr.)	
Author	Plant System	Maximum	Minimum
Mason & Lewin	Yam tuber	4·4	—
Clements	*Kigelia* fruit	2·6	—
Colwell	Squash fruit	9·5	1·7
Crafts & Lorenz	„ „	3·8	0·85

On the basis of these figures, a reasonable estimate for the mass-transfer in the vine systems used might be 0·5–1 g. per cm.^{2} of phloem per hour, since the petioles and stems are almost certainly less active at transporting carbohydrate than the fruit peduncles considered by the above authors.

Nothing can be deduced about the rate of transfer of sucrose through the experimental vine organs from the measurements reported in this paper once the steady state has been established of sucrose-^{14}C movement through the chamber, since, by the nature of steady states, rates of transfer are only accessible from measurements at the ends of the system, while we have been measuring a side reaction. The only clue to the rate of transfer lies in the rate of establishment of the steady state, and to relate the two we must assume some mechanism by which the establishment occurs. Our ignorance of the process is too great to allow our imagining a detailed physiological model whose mathematical analysis would give us a relation between the rates of transfer and establishment on which we could rely. Nevertheless, a simple assumption about the mechanism yields us a concrete relation which may be of use in the present dilemma and which is capable of further experimental testing. Assume that the time taken for the steady state to become established in a petiole is equal to the time for the total phloem sucrose within the chamber to be displaced by fresh sucrose from the leaf. The first can be

estimated from curves such as Fig. 1; the second is compounded of the masses and dimensions already measured and the rate of transfer we are seeking.

We have now reached a point in building an edifice of assumptions on the actual data when the errors involved in our assumptions far outweigh the errors in the measurements, rendering it useless to perform detailed operations on the individual measurements, but still useful to employ their average to give us orders of magnitude. Consider an average petiole containing phloem of cross-sectional area 0·7 mm.^{2}, whose sucrose content is 3 mg. (average of sucrose at site in the 'Probable breakdown' column of Table 3). Inspection of Fig. 1 and other curves of the same type allows us to estimate the time of establishment of the steady state as 7 hours. Now if this is the time taken to displace all the sucrose instantaneously present from our average petiole, the mass transfer must be 0·06 g./cm.^{2} of phloem/hr., which is lower by a factor of 10 than the estimate based on measurements in the literature, and lower by a factor of 100 than the maximum rates measured in fruit peduncles. None the less, it could still be a good estimate of the rate in the vine petiole, especially one whose stem is not attached to a root system, and we may take it as a minimum rate of sucrose transfer, with a round 1 g./cm.^{2}/hr. as the maximum.

It is pointless to compare our estimated breakdowns of sucrose in individual experiments with this rough estimate of translocated sucrose, so again we take the average of the probable breakdowns in the petioles of Table 3, 147 μg./piece/7 hr. In seven hours, the maximum sucrose transfer through a petiole is 49 mg., and the minimum, 3 mg.; and the average breakdown measured is 0·3 per cent. of the maximum and 5 per cent. of the minimum. On either estimate, the loss in transit is a small part of what is moved and the efficiency of the process must be rated highly. The stem system is so remote from the source of the sucrose-^{14}C that our basic assumption cannot be expected to apply.

General Considerations

We have not unequivocally answered the question of where the energy comes from to drive the translocation machine. We have fixed an approximate figure for the loss of translocated sucrose, but have no evidence that any of this is being applied to the translocation machinery or that other substrates outside the phloem are not being used to drive the process. Nevertheless, it has been firmly established that some of the translocated sucrose is respired in transit, and further, that this is a constant proportion of what is instantaneously present in the phloem, and of what passes through it. This is suggestive of an orderly rather than a random process, and it is proposed, as a working hypothesis, that we may regard the translocated sucrose as the substrate that supplies the energy for translocation, and that the quantity of this energy is that produced by the respiration of about 0·5 mg. of sucrose per c.c. of phloem per hour under the conditions of these experiments.

We can now compare this measured energy expenditure with the corrected energy requirement of Mason *et al.*, 0·004 g. of sucrose per c.c. of sieve-tube sap per day. If we assume, as many others have done, that the sieve-tube lumina occupy one-fifth of the phloem, the probable loss of sucrose estimated in these experiments is about 0·05 g. per c.c. of sieve-tube sap per day, or twelve times that necessary to maintain the streaming rate implied by the corrected calculation of Mason *et al.* The process of application of the energy need only be 8 per cent. efficient and yet proceed without using any other respiratory substrate than the translocated sucrose. We cannot argue from an energy expenditure back to a mechanism, any more than we can calculate energy expenditure without postulating a mechanism, but at least we are in the stronger position of knowing the energy expenditure, and may eliminate postulated mechanism that would require more energy.

The question of where the measured breakdown is going on is an interesting one, but on it the present work gives no information. If, as Wanner (1953) found for *Robinia*-phloem exudates, the vine sieve-tubes contain few of the glycolytic enzymes, the respiration of the translocated sucrose must be going on outside the sieve-tubes. Ziegler (1956) envisages some such system of energy supply: '. . . die Energie, die zur Aufrechtähnlicher Leistungen der Siebröhren notwendig ist, von den Geleitzellen, etwa in Form von ATP, den Siebröhren zugeführt wird'. The substrate for the respiration would of course have first to pass out of the sieve-tubes and into the companion cells. On the other hand, it can always be objected that the exudates studied by Wanner do not represent the full contents of the sieve-tubes, or even if they do, that the mechanism may be different in the vine. Ziegler, later in the paragraph quoted above, suggests that we have to deal with several different mechanisms of phloem transport in different plants, but until we are driven by the evidence to accept this view, 'nihil multiplicandum praeter necessitatem'. If the breakdown of translocate revealed in these experiments is indeed going on in trees, it may provide an explanation of the results of Zimmerman (1958).

Two facts are revealed in Zimmerman's work about the phloem exudates from *Fraxinus americana*: the first, that there is a fall in total sugar concentration of the exudate with distance down the trunk, amounting to 17 per cent. in 8 m.; the second, that if a tree is defoliated in summer, the sugar concentration of the exudate falls rapidly for the first day or two, the rate of fall being about 1·3 per cent. per hour. Zimmerman interprets the latter fall as leakage of sugars from the sieve-tubes, and imagines it as part of the normal removal of sucrose to other tissues which is going on all the time, and whose magnitude is revealed when the supply is cut off. In the vine there is a respiratory loss of sucrose in the phloem of 5 per cent. in 7 hours, or 0·7 per cent. per hour. Allowing for the physiological differences between a vine petiole and the trunk of an ash tree, it seems very likely that a large part of the disappearance of sugar from the ash exudates after defoliation is due to the continuing of respiration which was going on in the phloem before

defoliation. On this view, the negative concentration gradient downwards may be due to progressive loss by respiration with distance from the source of sugar, and the sugar concentration gradient would be a consequence, not a cause, of the translocation process.

Summary

1. $^{14}CO_2$ was supplied to vine leaves and as the labelled assimilate passed through the petiole away from the lamina, the atmosphere outside the petiole was sampled for $^{14}CO_2$. The breakdown of labelled sugar in the petiole is shown to be measured by the counts at infinite thickness of the carbonate precipitated from the sampled gas.

2. The time course of the rate of breakdown shows that a steady state is established 7–10 hours after application of the $^{14}CO_2$ and is maintained for about 3 days.

3. The only source of $^{14}CO_2$ in the petiole is sucrose-^{14}C in the phloem, and the collected $^{14}CO_2$ from outside the organ bears a constant ratio to the instantaneous sucrose-^{14}C content of the organ. About 5 per cent. of the instantaneous sucrose-^{14}C content is lost in 7 hours.

4. The corresponding respiratory loss of sucrose in the phloem is estimated to be about 200 μl. CO_2/g. fw./hr. This is apparently rather below the rate of respiratory CO_2 output for the rest of the organ.

5. Calculations of the probable quantity of sucrose translocated show that the loss in transit is between 0·3 per cent. and 5 per cent. of what passes through the petiole.

6. The energy expended is four times what was calculated to be necessary to drive a proposed 'streaming mechanism'.

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EXPLANATION OF PLATES

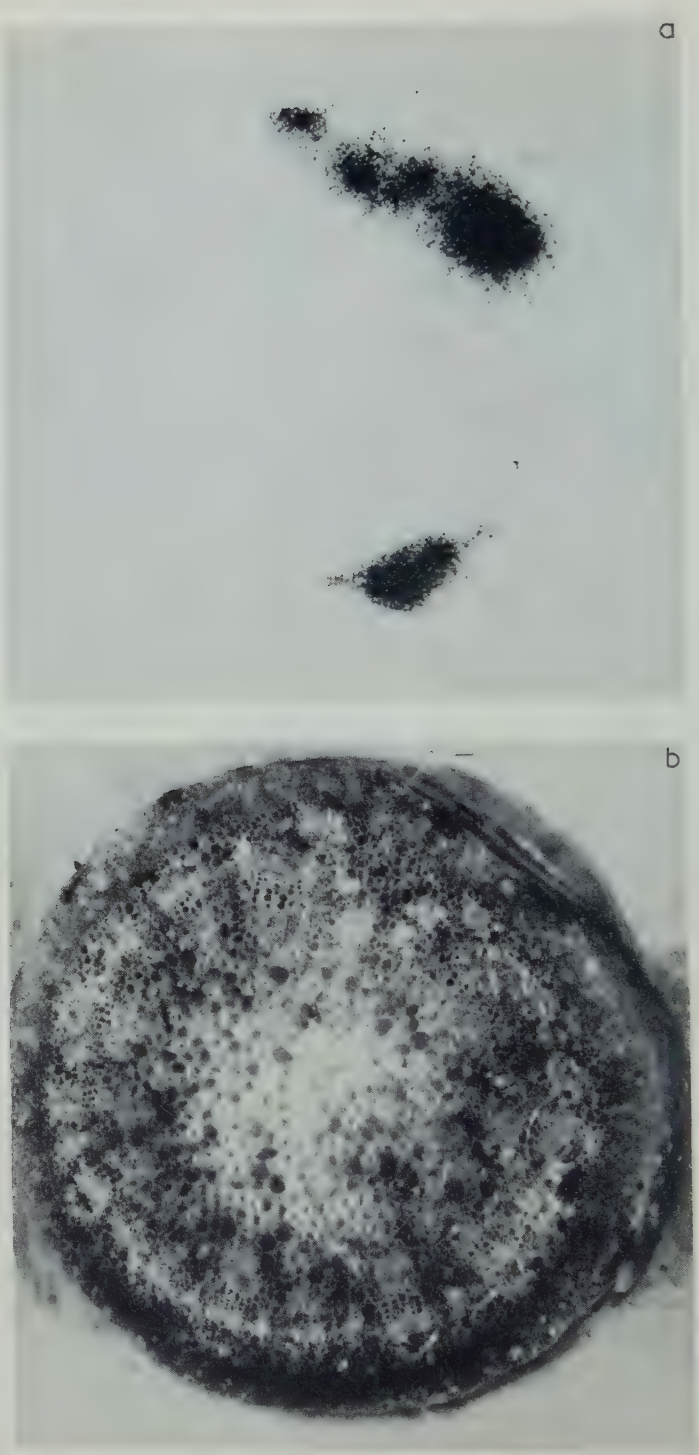
PLATE 1

The bottle for the application of $^{14}CO_2$ is sealed to the under surface of a leaf and the respiration chamber is sealed around the petiole. All $^{14}CO_2$ is lost from the bottle in an hour or two of photosynthesis, but until then the gas stream through the respiration chamber can be contaminated from the application $^{14}CO_2$ if there are leaks in both bottle and chamber, since there is a slight negative pressure in the chamber.

PLATE 2

Section (*a*) and radiograph (*b*) of petiole 3 of Table 2, showing the distribution of labelled sucrose in the petiole at the time of sampling.

M. J. CANNY



M. J. CANNY

The Effect of Number of Shoots on the Quantity and Distribution of Increment in Young Apple-trees

BY

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With three Figures in the Text

Abstract

It is shown that the shoots of a young tree act additively rather than competitively, that is, each extra shoot produces a definite extra increment in total weight, not that a constant increment is divided among the shoots of the young tree.

One-year apple-trees of 3 varieties were rigorously selected for size and weight, and in the spring disbudded to 1, 2, 3, or *n*, the natural number of shoots per plant. The treatments were applied in the 1st or 2nd year after planting and the increment during the treatment year was determined. Provided not more than a few secondary shoots were produced on the current shoots, each additional primary shoot produced an increase in weight. In one variety plants with 1 primary shoot produced many secondaries and these plants were heavier than those with 2 primaries.

The largest shoot was not greatly affected in size by the presence of other shoots except in the variety which produced secondary shoots readily. These were most abundant on plants with 1 primary shoot, fewer on plants with 2, and almost negligible on plants with 3 and *n* shoots.

Treatment and varietal effects on the percentage distribution of increment as leaves, new stem, old-stem increment, and root, were small. The percentage of leaves in the 2nd year was about half what it was in the 1st year; the percentage of root was nearly the same in both years.

Introduction

IN experiments with young trees it often happens that, despite very careful selection of the planting material, varying numbers of the sprouting buds grow out into extension shoots. How far this introduces additional variation into the experiment does not appear to have been investigated, apart from a brief note by Knight (1926). Sometimes this variation is avoided by rubbing out all but a designated number of the shoots, say, 1 or 2; alternatively the trees are left untouched, it being assumed that the experimental layout will average such effects over the treatments. In interpreting the results of an experiment on the interaction of rootstock and scion, where differing shoot numbers were part of the treatment effects, it became necessary to determine just what was this effect.

Materials and Methods

The method used was straightforward. Plants of known weight were allowed to develop 1, 2, 3, or *n* shoots; *n* was the number of shoots that grew out without disbudding. After a season's growth they were dug up and

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weighed. It was thought probable that the effect of shoot number might, in the 1st year, be different from subsequent years owing to establishment effects; the experiment was therefore carried on for 2 years. Plants due to be harvested at the end of the 1st year were disbudded appropriately after budbreak in the first spring; those to be harvested in the 2nd year were all disbudded to 2 shoots the first year, the new wood pruned off the following winter, and the appropriate number of buds allowed to grow out the second spring; 1 cm. of the uppermost new shoot of the 1st year was left with the object of providing a number of young buds to grow out, but in fact buds grew freely from the old stem. The plants of each variety were planted in pairs, one for each year. As a result of variations in shoot growth in the 1st year, the plants in the 2nd year were not so uniform when the treatments were applied as in the 1st year. It was hoped to minimize the effects of this variation by measurements on the weight and lengths of the prunings.

Varieties. Varieties differ considerably in their ability to produce side shoots on the current season's growth. Such side shoots will be called *secondary* shoots, in contrast to the *primary* shoot growing straight from a dormant overwintered bud. A variety freely producing secondaries might not be so 'restricted' in its growth, on being disbudded to 1 primary shoot, as one doing so sparingly. To get some measure of this effect, three contrasted rootstock varieties were included in the experiment.

i. M.XXV Stoolbed shoots average length, secondaries restricted to axillary spurs.

ii. 3426 Stoolbed shoots rather short, with a few secondaries.

iii. 3438 Stoolbed shoots above average length, secondaries often produced.

Selection and layout. Large numbers of rooted shoots were screened and only undamaged straight shoots within a narrow range of length and diameter accepted. These shoots were subsequently weighed and separated into groups of the same weight to the nearest gram. For each variety replicates of the treatments for a block came from within a single group. The shoots were then pruned to 70 cm. and reweighed; a sample was taken for dry weight. Distribution of increment between new stem and old stem might have been more uniform had the shoot been pruned by the same fraction (Maggs, 1959), but fortunately, owing to the rigorous selection limits, there would have been very little difference between the two methods. There were 16 replicates of each treatment.

Growth during the 1st year was not good, and considerable damage was done during budbreak by attacks of the clay-coloured weevil, *Otiorrhynchus singularis* L. In the 2nd year growth was satisfactory and disease-free.

Results

In presenting these results the main effects on increment will be described first, then the development from budbreak leading up to these effects, and finally the various interrelations of the parts.

Increment in weight. The estimated increments are shown in Fig. 1. The mean increments for the 1st year were calculated by subtracting the initial weight at planting from the final weight, and for the 2nd year by subtracting from the final weight the mean weight of the 2-shoot treatments sampled after 1 year less their weight of leaves and 1st-year shoot. The weight of the pruning stub was estimated to be 0·5 g. for all trees. A more statistical approach gave very similar results. This involved calculating the regression for

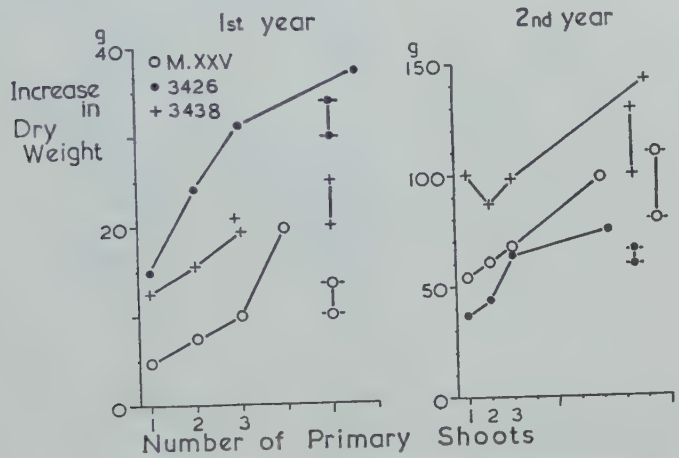

Fig. 1. Increments in weight of plants disbudded at the beginning of the 1st year (left); and at the beginning of the 2nd year (right). These later plants were all disbudded to 2 primary shoots in their 1st year. The vertical lines represent the Significant Difference between 2 treatment means for each variety ($P = 0{\cdot}05$).

the 1st year of New Shoot on Root plus Old Stem Dry Weight, and then applying this regression to the weight of prunings (New Shoot) for the trees sampled in the 2nd year. The values for increment so calculated were for all treatments between 5 and 10 grams lower than those shown in the figure; this is attributed to an increase in the weight of the trees in the autumn after the 1st year's sampling and before the trees were pruned.

In the 1st year the mean increment was only 18·2 g. dry weight per tree on an initial 13·8 g. as against 76·8 on an initial 21·3 g. in the 2nd year. Nevertheless treatment effects were similar in both years (Fig. 1). With the exception of variety 3438 in the 2nd year, the effect of each additional shoot was an increase in the final weight. The general trend over all varieties appeared to be linear, for while in individual cases the response to shoot number appeared curvilinear, the deviations from linearity were not consistent from variety to variety. The exceptionally high increment of 3438–1 shoot in the second season is ascribed to its profuse production of secondary shoots. The varieties were not in the same order of increment each year. In the 1st year 3426 grew fastest, followed by 3438 and M.XXV at roughly equal intervals, while in the 2nd year 3438 grew much faster than the other varieties.

Development of leaf area. The leaf areas of all plants were measured photometrically at the final harvest (Maggs, 1957*a*). On four occasions during each season's growth leaf areas of 5 out of the 16 blocks were estimated, every 3rd leaf being measured with Freeman and Bolas's grid (1956), starting from the base of the plant. The uppermost measured leaf was marked on each occasion so that the same older leaves were measured. The development of leaf area is shown in Fig. 2; a logarithmic scale is used to show the early details adequately. With a few exceptions, each additional shoot resulted in a stepwise

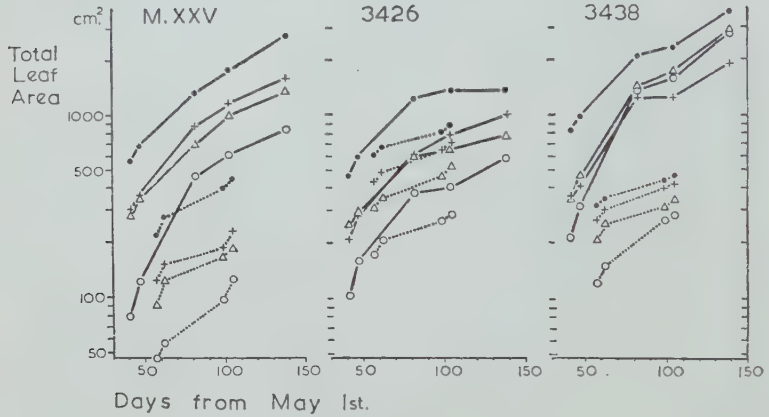

FIG. 2. Growth of total leaf area. Broken line = growth in the 1st year; solid line = growth in the 2nd year; ○ = 1 shoot, △ = 2 shoots, + = 3 shoots, ● = *n* shoots. The area scale logarithmic.

increase in leaf area. Such differences were established within 60 days from the beginning of leaf expansion and persisted throughout the growing season. In the 1st year the trees were sampled rather early, but the evidence from other experiments indicates that there would have been no marked changes between treatments had the trees been left longer. The crossing over of the lines representing 2- and 3-shoot treatments of the variety 3426 in the 2nd year may be ascribed to random abnormalities in budbreak, but in variety 3438 the rapid increase in leaf area of the treatments with 1 and 2 shoots is due to their early and continued production of secondary shoots.

Contributions of individual shoots. If the foliage of the different-sized shoots on a tree be assumed to function with equal efficiency, then the contribution that each shoot makes to the total increment may be measured by the duration and amount of its foliage. The area of the leaves on the longest shoot plotted against total leaf area on the tree on successive occasions was found to be closely linear for all varieties and treatments. It was therefore assumed that a comparison of the leaf areas of successively larger shoots late in the summer would give a measure of their assimilation during the season. Since it has been found (unpublished work) that the longest shoots have largest leaves and therefore the greatest leaf area, whether or not they have more leaves, shoots

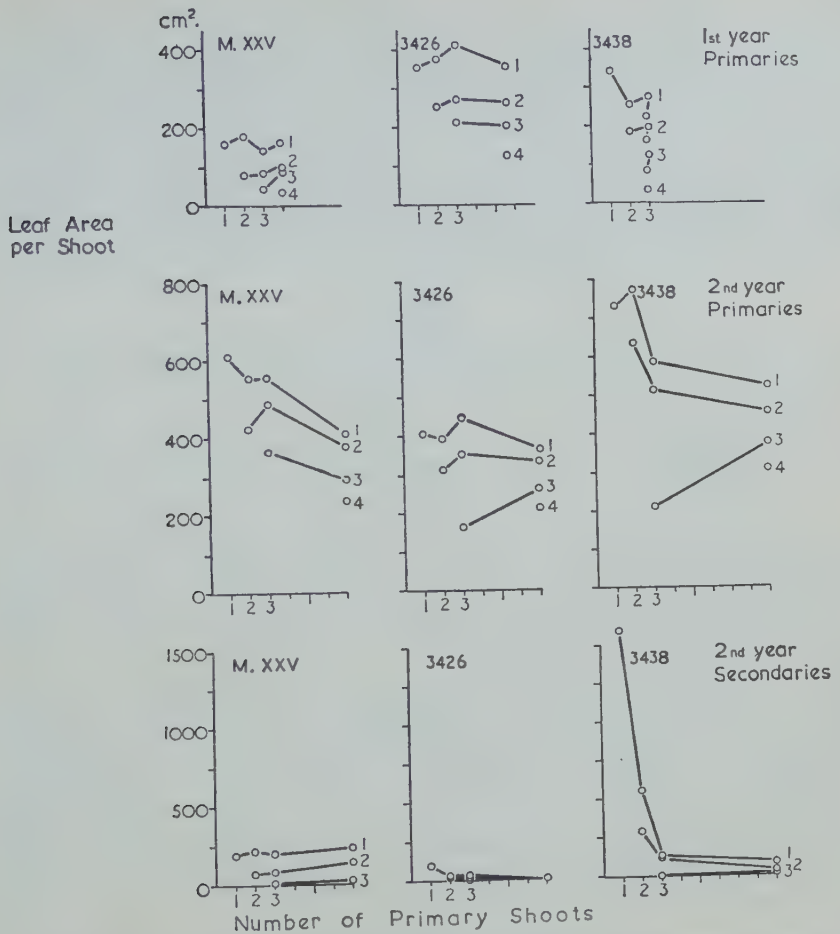

FIG. 3. Total leaf areas of longest (1), second longest (2), third longest (3), and fourth longest (4) shoots. There were very few secondary leaves in the 1st year.

were classified on the basis of their length, and the leaf areas of the longest, second, third, and fourth longest primary shoots were determined (so far as the treatments permitted) for each tree. Spurs were included, if necessary, in these calculations. These areas are shown in Fig. 3 for primary leaves, and for secondary leaves in the 2nd year. It is clear that the area of primary leaves on the longest shoot is but little depressed by the presence of other shoots on the plant; and the shoots of other ranks are similarly little affected by their neighbours. There is no definite evidence of competition or synergism between shoots once they have started to grow.

Secondary leaves. In the 1st year secondary leaves were negligible, but in the following year with established plants they profoundly modified the behaviour of variety 3438 (Figs. 2 and 3). In this variety by September, on plants with a single shoot, the area of secondaries was over twice the area of the

primaries; on plants with 2 shoots the secondaries were much less—on the largest shoots three-quarters of the area of the primary leaves, and on the second largest only half; while on plants with 3 and *n* shoots they accounted only for a tenth and a twentieth of the total area respectively. The relative importance of the secondary leaves to the development of the assimilating surface may be judged from the data on Leaf Area Duration in Table 1. These figures were obtained by plotting leaf area against time and planimetering the area under the resulting curves.

TABLE 1

Leaf Area Duration (1000 cm.² leaf-days)

Variety 3438

Number of shoots	1	2	3	9
Primary leaves	33·8	60·2	59·3	132·3
Secondary	51·3	31·4	11·2	3·3
Total	85·1	91·6	70·5	135·6

In the other two varieties the secondary leaves were much less important, the LADs for the secondaries ranging, for M.XXV, from one-fifth (1 shoot) to one-tenth (4 shoots) of the total, and for 3426 from one-fifth to one-fiftieth.

Other shoot measurements. Shoot length, leaf number, and mean area per leaf were all similarly affected by shoot number in both years. Total stem length and number of leaves increased with shoot number, while the length of the longest shoot tended to remain constant for any one variety. The mean area per leaf decreased as shoot number increased, while the longest shoots of a variety all tended to have leaves of the same area, those of the next longest being a little smaller and so on.

Distribution of Increment

Differences between treatments in the distribution of increment to leaves, stem, and root may be conveniently separated from the effect of size by expressing the results on a percentage basis. This method assumes that any effect of size on percentage can be ignored. The 1st year's data were therefore examined to test this. It was found that the total increment (x) and the part increment (y) were related fairly well by an expression of the form $y = kx^a$ where k and a are constants. If $a = 1$, y/x is constant. An examination of the relationship between $\log x$ and $\log y$ indicated that a did not differ significantly from 1, except for roots where y/x tended to decrease as x increased, and for New Stem where y/x tended to increase as x increased.

With these limitations in mind, the data of Tables 2 and 3 show some remarkable constancies in the percentage distribution of increment. In Table 2 differences of less than 5 per cent. may be considered non-significant and in Table 3, 3 per cent. The most obvious change was the drop in the percentage of leaf in the 2nd year to half its value in the 1st year. This was

shown by all treatments and varieties. Variety had little influence on the distribution; owing to the smallness of some increments the 1st year, there was a greater range in the values for each part. It might have been expected that the considerable production of secondary shoots in variety 3438 would have resulted in some abnormalities in stem or leaf percentage, but the evidence is against this. The production of shoots along the length of the old stem in the *n* treatments meant that the lower shoots had less of the old stem to thicken, and this is reflected in the lower percentage for old-stem increment in the 2nd year.

TABLE 2

Percentage Distribution of Increment during the Experimental Year between Leaf, Stem, and Root

	M.XXV				3426				3438			
No. of shoots	1	2	3	*n**	1	2	3	*n**	1	2	3	*n**
	1st year (1956) Sampled 22 August											
Percentage of increment as:												
Leaves	39	48	40	31	36	35	36	40	38	35	35	34
New stem	24	20	17	16	18	17	17	17	21	16	17	16
Old-stem increment	19	13	28	36	31	33	32	28	29	34	33	37
Root	18	18	15	16	14	15	14	16	12	14	13	13
Mean value of *n**				3·9				5·6				2·9
	2nd year (1957)† Sampled 26 September											
Leaves	18	16	19	21	19	21	19	25	19	19	19	21
New stem	23	25	22	23	16	19	21	27	24	24	22	25
Old-stem increment	46	45	45	41	48	48	45	34	44	44	45	37
Root	14	14	14	17	18	12	15	14	13	13	14	17
Mean value of *n**				7·0				7·3				9·0

* The number of shoots on undisbudded plants.

† All plants grown with 2 shoots in 1st year and all but 1 cm. of these shoots cut off in the winter.

DISCUSSION

The longest shoot was of practically the same size in all treatments, as were the second and third longest shoots. Apparently there was no competition between the shoots; but there was some inhibition of the smaller shoots by the larger. This is in line with the results reported by Knight (1930) and Hatton and Amos (1927), who showed that the presence or absence of side shoots (feathers) on the main stem of young trees did not affect the growth of the leader. Books on fruit-tree raising sometimes use the concept of canalizing the growth of the young tree into a few branches, e.g. by pruning. This may be fallacious, and a better interpretation would seem to be in terms of relative amounts of new- and old-stem increments as a response to pruning (Maggs, 1959). The result also has a bearing on the techniques of experimenting with young trees. Where quantities such as weight increments, or amounts of extension growth, are important, it would seem to be essential to start the experiments with the various treatments all carrying the same number of new shoots, or to allow for variation in shoot number statistically.

Had the trees been grown on for several years, it is likely that the differences between treatments would have decreased, but not disappeared. In single-stemmed trees buds will grow out most of the way down the stem, but on trees with several stems budbreak will be confined to the tips of the shoots, so that the tendency will be for all treatments to have the same total number of new shoots. The course of development will gradually become similar in all treatments, save that those with most shoots originally will be a year or two ahead of those with fewest. Thus, Knight (1926) found that in the 1st year

TABLE 3

Percentage Distribution of Increment—Averages for Treatment and Variety

	1st year				2nd year			
No. of shoots	1	2	3	*n*	1	2	3	*n*
Leaves	38	39	37	35	18	20	19	22
New stem	21	17	17	16	20	21	21	24
Old-stem increment	26	28	31	34	47	45	45	37
Root	15	16	15	15	15	14	15	17

Variety	M.XXV	3426	3438	M.XXV	3426	3438
Leaves	39	37	36	20	20	19
New stem	19	17	17	22	19	22
Old-stem increment	25	31	34	43	45	43
Root	17	15	13	15	16	16

multiple-budded shoots totalled 180 per cent. of the new growth of single-budded plants, while by the 3rd year they totalled only 120 per cent. A similar evening-up in growth has been reported by Chandler (1923) for young peach trees.

The behaviour of the variety 3438 in the 2nd year shows that where secondary shoots are readily produced they can overcome the limiting effect of low primary shoot number. Such an effect can only be manifest under good growing conditions; thus the variety 3426 produced few secondaries and increments increased with shoot number, whereas in an earlier experiment (Maggs, 1957*b*) where growth was very vigorous, abundant secondaries were produced, and under such conditions the response to shoot number might have approximated to that of 3438. On the other hand, a variety such as Blenheim Orange very rarely produces more than a few secondary spurs, however lush its growth.

The main differences between the amounts and distribution of increments in the 1st and 2nd years are probably reflections of the better growth made in the 2nd year when the trees had established themselves. Thus although the actual weight of leaf rose from 7 to 15 grams in the 2nd year, the leaf percentage fell from 37 to 19 per cent., so that the production of increment in terms of leaf weight must have nearly doubled.

On the other hand, the percentage of root remained almost constant at 15 per cent., strongly suggesting that it is within the root-system that the limitation of growth has arisen, in agreement with the conclusions from the

previously mentioned pruning experiment. The root in the 1st year was all new root, while in the 2nd year part of the increment was formed as secondary thickening on the 1st year's root system and part was extension of the root system. Therefore if the form of the root system remained constant, in the 2nd year there was a much greater amount of old root to be thickened and consequently a smaller proportion of the increment would have been in the form of new extension. Therefore if the proportion of new absorbing root tips had remained constant, either the form of the root must have changed, e.g. by death of the smaller roots, or the weight of new absorbing roots must in both years have been a very small amount. Rogers (1939) and Kolesnikov (1930) have reported the death of fine roots in the soil during the current year and this has also been observed in young rootstocks growing in transparent plastic bags. There is also evidence that the proportion of fine root (less than 1 mm. in diameter) decreases with greater increment. Thus Knight and Hoblyn (1934) showed this to be so with young trees of Grenadier apple on various rootstocks after growing unpruned for 2 years; in this case the percentage of fine root declined to a minimum value of 6 per cent. Rogers and Vyvyan (1928) found that the percentage of fine roots in some 10-year-old trees varied from 6 to 14 per cent. and suggested that this variation was due to differing mortality of fine root in different years. It seems probable that there is a change of form of the root system with age (or size) reaching eventually a more or less stable equilibrium. This change in form might be brought about by a change-over from a pattern of growth where new root-branch systems are developed from the tips of the major roots to one where these systems developed from laterals arising back in the old lignified root system. The extreme contrast would be between a raceme and an umbel. Root-systems of both these kinds have been observed in the somewhat abnormal conditions of water- and mist-culture, and it is well known that differences in soil aeration can markedly affect the amount and kind of new roots. Vyvyan (1930) has described how the balance between terminal and basal production of new root may be altered by rootstock and scion variety. This equilibrium in the partition of increments between secondary thickening and new root extension growth might vary according to the conditions of growth and nutrition of the whole plant. Finally, a reduction in the proportion of apical meristems in the root increment might well be correlated with a reduction in the proportion of leaves.

An interesting possibility suggested by this experiment is the determination of an expression for the growth of the tree. If the increment is related to the number of shoots, the problem is resolved into determining the time course of (*a*) shoot number, and of (*b*) the relation between shoot number and increment. Such a wide extrapolation from this experiment is somewhat speculative, and data covering 8 years' growth from the experiment described by Vyvyan and Maggs (1954) are being examined to determine the feasibility of the idea.

Summary

1. Three varieties of apple rootstock were selected within close limits of size, and allowed to develop 1, 2, 3, or *n*, the normal number of shoots per plant. The treatments were applied in the planting year, or in the year after planting. Trees were weighed at the end of the treatment year.

2. Each additional shoot resulted in an increase in the final dry weight. The exception was variety 3438 in the 2nd year, where the 1-shoot treatment was heavier than the 2-shoot treatment. The variety putting on most weight in the 1st year did not put on most weight in the 2nd.

3. Where secondary shoots were not freely produced, there was only slight competition between the shoots. Thus, within a variety and year, the longest shoots were of approximately the same size irrespective of the number of shoots on the tree, as were the second longest and the third longest.

4. Where secondary shoots were freely produced (var. 3438—1 shoot in the 2nd year) the increment was as great as that produced by treatments with more primary shoots.

5. In the 1st year within treatment and variety the percentage new stem/total increment significantly increased as increment increased, and of root/total increment decreased as increment increased.

6. The percentage distribution of increment to leaf, new stem, old stem, and root was not affected by number of shoots or variety in either year. The percentage of leaf was much lower in the 2nd year, while the percentage of root was nearly the same in both years.

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Variations with Age in Net Assimilation Rate and other Growth Attributes of Sugar-beet, Potato, and Barley in a Controlled Environment

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With two Figures in the Text

Abstract

Sugar-beet, potato, and barley plants were grown in a controlled environment, for periods of up to 10 weeks from sowing, with a light intensity of 1,800 f.c. (4·9 cal./cm.^{2}/hr.) and a temperature of 20° C. during the 18-hour photoperiod and 15° C. during the dark period, to test whether net assimilation rate varied with age and differed between the three species.

Net assimilation rate of all species based on leaf area (E_A) fell approximately linearly with time. During 5 weeks E_A of sugar-beet decreased by only about 20 per cent. and E_A of potato decreased by 50 per cent. E_A of barley remained approximately constant for 4 weeks after sowing and was halved during the subsequent 4 weeks. The average value of E_A for all times was greatest for sugar-beet and least for barley.

Net assimilation rates based on leaf weight (E_W) and leaf N (E_N) decreased at about 15 per cent. of the initial value per week for all species; this was similar to the mean rate of decrease of E_A of potato and barley, but greater than that of E_A of sugar-beet. Mean values of E_W or E_N for potato and barley were similar and less than for sugar-beet.

Relative growth rate (R_W), relative leaf growth-rate (R_A), and leaf-area ratio (F) fell with time at similar rates for all species. Average values of R_W decreased and of F increased in the order sugar-beet, potato, barley. R_A was greatest for potato and least for barley.

Introduction

NET assimilation rate (E) and other growth attributes of plants grown in the variable conditions of field or glasshouse show both short-term fluctuations and smooth trends with time. Both types of variation probably reflect changes in the environment but the smooth trends may be partially attributable to changes with age that are independent of the environment. It is difficult to distinguish the effects of age from seasonal trends in external factors and to estimate the magnitude of each. The effects on E of short-term fluctuations in environmental factors have been estimated using partial correlation coefficients. Gregory (1926) found that over 80 per cent. of the variation in E of barley up to the time of maximum leaf area could be accounted for by variation in external factors, and, as there was no obvious time trend in E, concluded that change with age was negligible. Such use of partial correlation is an uncertain and insensitive method for identifying environ-

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mental effects and separating them from trends due to age, because choosing and measuring the relevant external factors is difficult and because they are correlated with each other and with time (Williams, 1946).

A better method of determining whether growth attributes differ with age is to measure them in the same conditions on plants that have been sown at different times. In one such experiment E was similar for sunflower plants sown 18 and 28 days previously (Blackman and Wilson, 1951) and in another, delay in sowing of sugar-beet by 6 weeks increased E by an amount which was small compared with the seasonal trend attributable to change in external factors (Watson, 1947). This result does not prove that E decreased with age independently of environmental effects, because differences in the environment before the period when E was measured may have had a persistent effect.

The extent of specific differences in E is also doubtful. Valid comparisons are limited to species adapted to similar conditions and having similar growth periods because, to avoid environmental effects, E of different species must be determined at the same place and time and, to avoid age effects, it should be determined on plants of the same age. Taking these precautions, Watson (1947, 1952) found significant differences in E between several species and also found consistent differences between mean values of E determined at the same period of different years for different species grown in neighbouring fields. Blackman and Wilson (1951) also found significant differences in E between species of the same age grown in the same conditions.

Heath and Gregory (1938) concluded that E is nearly the same for all species and is constant during the vegetative phase. Although later work has produced evidence of variation in E both between species and with age, its extent and importance in determining variations in dry-matter production between plants is still questioned (Gregory, 1950). This controversy continues because of the confusion of inherent changes in growth with those caused by environmental differences and could be settled by experiments done in controlled environments where environmental factors can be held constant. Four such experiments, comparing the growth of sugar-beet, potato, and barley, are reported in this paper.

The temperature and light intensity in the experiments were a compromise between the optimum requirements of the three species concerned, in so far as these were known and could be satisfied by the facilities available. The day temperature selected was 20° C., the night temperature 15° C., and the light intensity was approximately 1,800 f.c., the maximum obtainable. An 18-hour light period was used to produce as large dry-weight increase as possible so that E and other growth attributes could be determined with maximal precision.

Methods

The experiments were done during 1958–9 in the controlled environment rooms of the Plant Research Institute, Canada Department of Agriculture, Ottawa.

Plants were grown under light panels consisting of 24 8-ft. (73 watt) standard cool white slimline fluorescent tubes and 10 60-watt tungsten lamps. The distance between the plants and the lights was adjusted weekly to maintain an intensity of approximately 1,800 f.c., measured with a Weston illumination meter having a quartz filter. This is equivalent to 4·9 cal./cm.^{2}/hr. in the wave band 360–700 mμ. The photocell of the light meter was held at the base of the youngest leaves of the barley plants and in the same plane as the youngest fully expanded leaves of the sugar-beet and potatoes. Temperature was set at the beginning of each experiment to 20° C. day temperature and 15° C. night temperature. Air temperature at the position where the light measurements were made was measured weekly with thermocouples. The average recorded light intensities and day and night temperatures in the 4 experiments at the positions stated were: Expt. 1: 1,776 f.c., 19·9° C., 14·5° C.; Expt. 2: 1,894 f.c., 19·5° C., 14·6° C.; Expt. 3: 1,773 f.c., 18·8° C., 14·6° C.; Expt. 4: 1,775 f.c., 18·8° C., 14·7° C. Relative humidity did not fall below 70 per cent. Air in the growth rooms circulated at 3 changes per minute and fresh air was introduced at 15 changes per hour, which had previously been found sufficient to prevent the depletion of CO_2 by photosynthesis.

One sugar-beet or potato plant, or 6 barley plants were grown in a 1-gallon glazed pot. Excess sugar-beet and barley seed was sown directly in the pots and the seedlings later thinned to the required number. Single eyes weighing about 2 g. were excised from potato tubers treated with 2-chloroethanol to break the dormancy that still existed in December, and sprouted in vermiculite or sand at 25° C. Sprouted eyes of a uniform size were later transplanted into the pots. The varieties used were: sugar-beet, Kuhn R; potato, Green Mountain in Expt. 1 and Up-to-Date in Expt. 2; barley, Brant.

Each pot contained a similar volume of sand or vermiculite irrigated with $\frac{1}{2}$-strength Hoagland nutrient solution, or 4·5 kg. of a mixture of 8 parts loam: 2 parts sand, to which 3 g. K_2HPO_4 and 3 g. NH_4NO_3 had been added in 25 ml. of solution. Water was added as necessary to maintain the soil at field capacity, as judged by its feel through a drainage hole in the side of the pot. For the sand treatment 24-mesh washed sand was put on top of a little 4-mesh quartz grit. Each pot was flooded 4 times in 24 hours with nutrient solution which entered and drained through the centre of the conical base of the pots. Similar pots were filled with a mixture of equal volumes of vermiculite and 6-mesh grit and about 500 ml. of nutrient solution was added to the surface twice a day (Went, 1957). The excess solution drained back to the supply tank. All nutrient solutions were renewed weekly.

Experiment 1. Sugar-beet, potatoes, and barley were grown in all combinations with soil, sand, and vermiculite. There were 3 replicates and 3 samplings. Sowing dates were staggered so that the initial dry weights of all species should be approximately equal. Sampling 1 was 20 days after sowing barley, 29 days after sowing sugar-beet, and 20 days after transplanting the sprouted potato eyes. Samplings 2 and 3 were 2 and 4 weeks later. A random arrangement of growing media and species was not practicable, so the media

were arranged in a 3×3 latin square. Within each of the 9 plots there were 3 sub-plots for each species, subdivided again for the 3 sampling occasions. On statistical analysis of the data, the 3 separate estimates of error required by this design did not differ significantly and a pooled error was used for all comparisons. N, P, and K content of the material from the final sampling was determined so that nutrient uptake from the 3 growing media could be compared.

Experiment 2. To amplify the information obtained from Expt. 1 about specific differences in time drifts of E, sugar-beet and potatoes were grown in irrigated sand and 6 replicates of each species were harvested on 6 occasions at weekly intervals. The first sampling was 19 days after sowing the sugar-beet and 11 days after transplanting the potatoes. The 2 species were grown in separate blocks to avoid shading should one grow taller than the other. Potato plants were allotted to each of the 6 replicates according to their initial size. Initial size differences among the sugar-beet were negligible and the plants were divided according to differences in mean light intensity measured before sampling 1. It was impossible to maintain exactly 1,800 f.c. for all plants and those at the edge of the light panel under a slightly lower intensity were included in the same replicates as the smaller potato plants. Nitrogen estimations were done on all leaf laminae in order to calculate E based on leaf N (E_N, see below).

Experiment 3. This was part of another experiment to be reported elsewhere comparing growth of barley in various conditions. Five pots of barley grown in soil were harvested on 4 occasions at fortnightly intervals, the first 25 days after sowing. Pots were allocated to each replicate according to mean light intensity before sampling 1.

Experiment 4. Conditions in this experiment were intended to be identical with those of Expt. 3 to obtain details of growth during 25 days following sowing. Six replicates were harvested on 4 occasions at weekly intervals, the first 11 days from sowing. Nitrogen content of lamina and sheath, separated from the underlying stem, was determined to calculate E_N. For this the 6 replicates from each sampling were bulked to make 2.

Harvest procedure. On each sampling occasion shoots and leaves were counted and fresh and dry weights of leaves, stems, and roots were measured. Leaf area of sugar-beet and potato plants was determined by rating each leaf in comparison with a set of standards of known areas, and summing the areas of all leaves (Williams, 1954).

Leaf area of barley was taken as one side of the lamina plus the outer surface of the sheaths and exposed stem (Thorne, 1959) and determined by measuring the length and greatest breadth of the laminae and the length and mean diameter of the green stems (Watson, Thorne, and French, 1958). Every shoot was measured in Expt. 4 and at sampling 1 of Expt. 3. Otherwise 1 plant per pot was measured and total area calculated from the ratio of leaf area to fresh weight of laminae and sheaths of the single plants, and the fresh weight of laminae and sheaths of the whole pot. The factor for converting

length by breadth of lamina to area was determined in each experiment, and varied from 0·74 to 0·78.

In Expts. 1 and 3 leaf area was also measured by a rating method similar to that used for field experiments (Watson *et al.*, 1958). On each sampling occasion a set of standard shoots was chosen from a spare pot that had been treated similarly to the experimental ones, all the shoots in each pot were rated on this scale and 36 or 24 randomly selected shoots were also measured. The areas of each class were obtained from a calibration curve calculated as the regression of log. leaf area on class number for the measured shoots. At each sampling the area of plants of the next sampling was determined as well as that of the harvested ones. To avoid bias in this comparison all shoots were rated in the pot.

Calculation of growth attributes. The method of Goodall (1945) was used to improve the accuracy of the dry-weight increments and mean leaf areas for the intervals between harvests (McIntyre and Williams, 1949; Watson and Wilson, 1956). At sampling 1 plants to be harvested at sampling 2 had their areas measured, and their dry weights at the time of sampling 1 were estimated from their areas and the regression of plant dry weight on leaf area, calculated from the plants of sampling 1. This procedure was always adopted except for barley of sampling 2 in Expt. 1, when the regression of dry weight on leaf area was not significant, and in Expt. 4, where the rating method was not used and so the area of unharvested plants could not be determined. In these cases the dry weights and areas of plants at one sampling were taken as the initial values for those harvested at the next sampling. Even with the greater uniformity obtained in a controlled environment, estimating initial dry weights from the initial leaf areas increased the accuracy of estimates of E, especially those obtained from single plants. For the first interval of Expt. 1 the standard error of E for a single pot was decreased from 19 per cent. to 12 per cent. of the mean for barley, from 26 per cent. to 12 per cent. for sugar-beet and from 35 per cent. to 7 per cent. for potato, compared with values obtained using independent samples to give initial and final weights and areas.

Net assimilation rate on a leaf-area basis, E_A, was calculated from the usual formula:

$$\frac{(W_2-W_1)(\log_e A_2-\log_e A_1)}{(A_2-A_1)(t_2-t_1)},$$

where W_2 and A_2 are the dry weight and leaf area of a plant harvested at the end of an interval (t_2); W_1 and A_1 are estimates of the dry weight and leaf area of the same plant at the beginning of the interval (t_1), obtained as described above; and t_2-t_1 is the length of the interval in weeks.

Net assimilation rates based on leaf nitrogen (E_N) and leaf weight (E_W) (Williams, 1946) were estimated in Expts. 2 and 4 by substituting mg. N in the leaves or dry weight of leaves per pot at beginning and end of the interval for A_1 and A_2 in the formula for E_A. In Expt. 2 the initial values of leaf N and leaf weight were calculated from the initial leaf areas in the same manner

as was total dry weight, using the regression of leaf N or leaf weight on leaf area. In Expt. 4 leaf N and leaf weight were those of lamina plus sheath, excluding stem.

Other growth attributes calculated were:

$$\text{Relative growth-rate } (R_W) \quad (\log_e W_2 - \log_e W_1)/(t_2 - t_1)$$

$$\text{Relative leaf growth-rate } (R_A) \quad (\log_e A_2 - \log_e A_1)/(t_2 - t_1)$$

$$\text{Leaf-area ratio } (F) \quad \frac{(A_2 - A_1)(\log_e W_2 - \log_e W_1)}{(\log_e A_2 - \log_e A_1)(W_2 - W_1)},$$

where W_1, A_1, W_2, A_2 represent dry weight and leaf area at beginning and end of an interval $t_2 - t_1$ weeks as in the formula for E_A.

Results

Experiment 1. The sowing dates were not sufficiently staggered to give equal dry weights per pot at sampling 1; that of barley was largest and sugar-beet smallest (Table 1). Barley continued to have the greatest dry weight and leaf area throughout the experiment but the differences between sugar-beet and potato were small, usually insignificant, and varied with the growing medium. At the final sampling sugar-beet had greater dry weight but smaller leaf area than potato.

Table 1

Expt. 1. Dry Weight and Leaf Area per Pot of 1 Sugar-beet or Potato, or 6 Barley Plants. Means for all Growing Media

Sampling	Dry weight, g./pot				Leaf area, dm.²/pot			
	Sugar-beet	Potato	Barley	S.E.	Sugar-beet	Potato	Barley	S.E.
1	0·88	1·28	2·90	0·145	1·55	1·89	6·50	0·328
2	10·5	9·0	22·2	1·05	13·8	12·5	53·4	1·08
3	43·7	33·5	59·5	2·86	30·5	39·0	122·3	4·48

At sampling 2 potatoes in all media had a terminal flower-bud and numerous side shoots, many of which also had flower-buds. Sugar-beet developed a large tap root that accounted for 32 per cent. of the total dry weight at sampling 3, but tubers accounted for only 2 per cent. of the total dry weight of the potato plants.

Net assimilation rates (E_A) of the 3 species differed significantly (Table 2). E_A of barley was always less than that of the other 2 species and E_A was less for potato than for sugar-beet in the second but not in the first interval. It decreased between the 2 intervals for barley and potato but not for sugar-beet. The high E_A of sugar-beet in the second interval resulted in a dry weight increment greater than that of potatoes though their mean leaf areas were similar, and relative leaf growth-rate (R_A) was similar for all species (Table 2). The high E_A of sugar-beet and the low one of barley were not caused by

differences in the ratio of photosynthesizing to respiring tissue; sugar-beet had the smallest and barley the largest leaf-area ratio (F). Relative growth-rate (R_W) was greatest for sugar-beet and least for barley and fell with time for all species.

TABLE 2

Expt. 1. Growth Attributes, Means for all Growing Media. Net assimilation rate (E_A), g./m.²/week; relative growth-rate (R_W), per cent./week; relative leaf growth-rate (R_A), per cent./week; leaf-area ratio (F), cm.²/g.

	Sampling 1–2			Sampling 2–3			
	Sugar-beet	Potato	Barley	Sugar-beet	Potato	Barley	S.E.
E_A	79	73	40	80	45	23	2·8
R_W	123	118	98	72	58	49	6·3
R_A	105	107	98	39	47	39	4·8
F	157	159	246	91	129	220	8·1

The differences between media were mostly small and insignificant. Early growth was best in vermiculite, perhaps because of its better moisture retention, and later growth was best in sand, probably because it supplied more nutrients (Tables 3 and 4). In sand, barley continued tillering up to sampling 3 to give a large leaf area.

TABLE 3

Expt. 1. Dry Weight, Leaf Area, and Shoot Number at Sampling 3 of 1 Sugar-beet or Potato, or 6 Barley Plants Grown in Sand, Soil, or Vermiculite

	Dry weight, g./pot			Leaf area, dm.²/pot			Shoot nos./pot
	Sugar-beet	Potato	Barley	Sugar-beet	Potato	Barley	Barley
Sand	46	39	66	34	49	175	92
Soil	42	31	51	30	38	106	68
Vermiculite	43	30	61	27	30	86	47
S.E.		5·0			7·8		6·9

TABLE 4

Expt. 1. Nutrient Content at Sampling 3 of 1 Sugar-beet or Potato, or 6 Barley Plants g. per pot

	N			P			K		
	Sugar-beet	Potato	Barley	Sugar-beet	Potato	Barley	Sugar-beet	Potato	Barley
Sand	1·49	2·02	2·72	0·26	0·30	0·45	2·87	2·30	3·89
Soil	1·29	1·15	1·53	0·17	0·10	0·15	1·52	1·22	1·63
Vermiculite	1·23	1·18	1·57	0·20	0·17	0·21	2·88	1·79	2·45
S.E.		0·202			0·026			0·230	

Growth in vermiculite had several unusual features. Ear emergence of barley was further advanced than in the other media. At sampling 3 the percentages of shoots having ears were 68, 15, and 4 respectively in vermiculite, soil, and sand. These differences in rate of development cannot be explained by differences in nutrient uptake from the 3 media. Uptake of N from vermiculite was less than from sand but the same as from soil, and K uptake was greater than from soil but less than from sand. K uptake by all species was high from vermiculite, which may have supplied some K in addition to that in the nutrient solution (Table 4). E_A of plants grown in vermiculite was significantly greater than of those grown in soil. The means for all species and both intervals were: vermiculite 61, sand 57, soil 52$\pm$2·0 g./m.^{2}/week. The differences were most noticeable in the second interval and applied to sugar-beet and potato as well as to barley, for which photosynthesis in the earlier emerging ears probably contributed to the higher E_A with vermiculite. The causes of earlier ear emergence and greater E_A in vermiculite compared with sand or soil are not clear.

This preliminary experiment showed that the 3 species grew satisfactorily in irrigated sand or soil and also showed the range of growth-rates obtainable in the selected controlled environment. This information was amplified in subsequent experiments in which sugar-beet and potato were grown in sand and barley was grown in soil. Soil had to be used for barley because Expt. 3 was part of another experiment in which sand culture was impracticable for reasons unconnected with the present investigation. To avoid confusion arising from differences between growth media, data from Expt. 1 referred to subsequently are those for sugar-beet and potato grown in sand and for barley grown in soil.

Experiment 2. Sugar-beet and potato. Dry weight per plant was the same for sugar-beet and potato until sampling 2, after which it was greater for sugar-beet. Sugar-beet had greater leaf area at the first 4 samplings but later the 2 species had similar areas (Table 5, Fig. 1). Potato plants had fewer side shoots than the variety used in Expt. 1. At sampling 5 there were side shoots in the axils of most of the leaves of the main stem, which had a terminal flower-bud. Leaves on side shoots accounted for 7, 30, and 43 per cent. respectively of the total leaf area at samplings 4, 5, and 6. Sugar-beet had a few axillary buds which contributed a negligible amount to the total dry weight and leaf area. At sampling 6 there were no potato tubers though stolons were well developed. Tap root of sugar-beet accounted for 26 per cent. of the total dry weight. Leaves were produced on the main stem of both species at a uniform rate of about 3 per week and less than 3 leaves per plant had died at sampling 6 (Fig. 1). Dry weight, and to a less extent leaf area, were slightly greater than in Expt. 1, possibly because the light intensity was higher (p. 358).

Net assimilation rates based on leaf area, leaf N, and leaf weight (E_A, E_N, and E_W) fell nearly linearly with time, but for sugar-beet the regression coefficient of E_A on time was small and not significant (Fig. 2). E_A of sugar-beet fell to only 80 per cent. of its initial values in 5 weeks, while the others

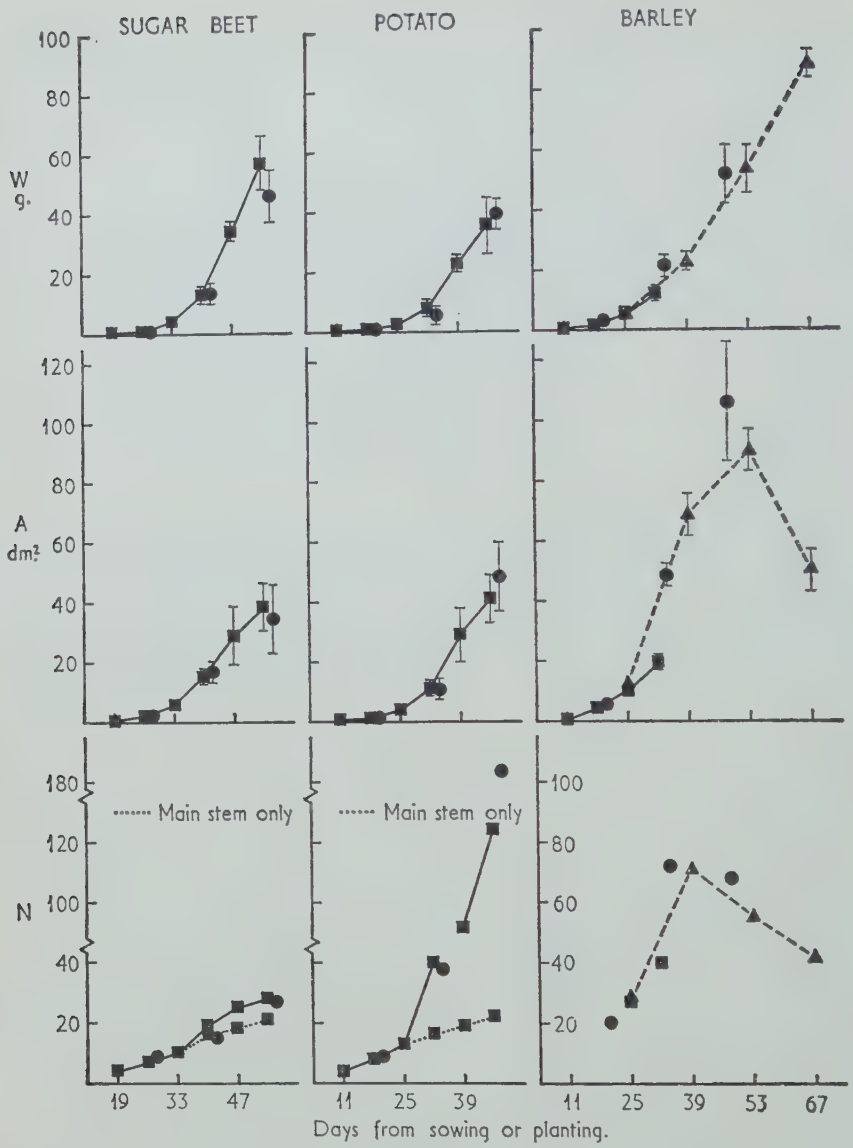

FIG. 1. Dry weight (W), leaf area (A), leaf or shoot number (N) of 1 sugar-beet or potato, or 6 barley plants per pot.
● Expt. 1, ■ Expts. 2 and 4, ▲ Expt. 3.
Vertical lines show 5 per cent. fiducial limits of each mean.

fell to about 50 per cent. of their initial values. E_N and E_W decreased at the same rate for sugar-beet and potato. The regression coefficients of E on time were: E_A: sugar-beet $-4{\cdot}6\pm2{\cdot}4$, potato $-18{\cdot}0\pm2{\cdot}0$; E_N: sugar-beet $-6{\cdot}39\pm0{\cdot}82$, potato $5{\cdot}30\pm0{\cdot}56$; E_W: sugar-beet $-0{\cdot}395\pm0{\cdot}040$, potato $-0{\cdot}448\pm0{\cdot}039$. E_A, E_N, and E_W were greater for sugar-beet than for potato

except in the first interval, when the difference was not significant for E_W and reversed for E_A.

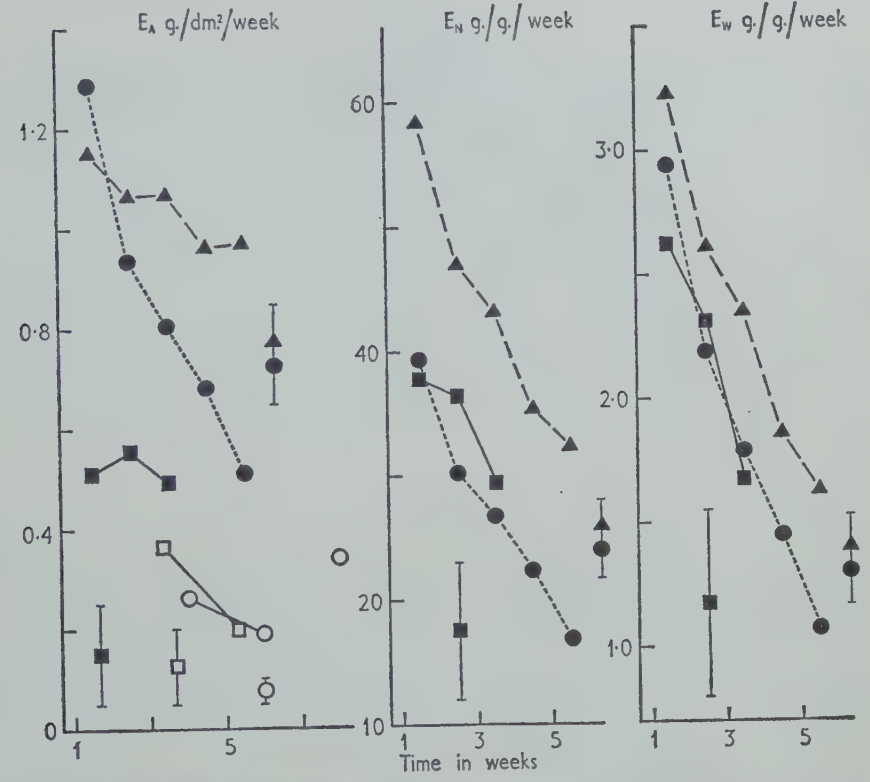

FIG. 2. E_A, E_N, and E_W of sugar-beet (▲ Expt. 2), potato (● Expt. 2), and barley (□ Expt. 1, ○ Expt. 3, ■ Expt. 4).

Week 1 was 19 days from sowing sugar-beet and 11 days from sowing barley or planting potatoes.

Least significant differences ($P = 0{\cdot}05$) appropriate to each experiment are shown.

TABLE 5

Dry Weight and Leaf Area of Sugar-beet and Potato (Expt. 2, 1 plant per pot) and Barley (Expt. 4, 6 plants per pot) at Weekly Intervals. Sampling 1 was 19 Days after Sowing Sugar-beet and 11 Days after Sowing Barley and Planting Potatoes

	Sampling 1			Sampling 2			Sampling 3		
	Sugar-beet	Potato	Barley	Sugar-beet	Potato	Barley	Sugar-beet	Potato	Barley
Dry weight, g./pot	0·09	0·15	0·26	0·69	0·71	1·23	3·75	2·45	4·86
S.E.	0·021		0·005	0·073		0·083	0·249		0·423
Leaf area, cm.²/pot	19	14	60	126	106	417	547	380	1008
S.E.	2·9		1·6	13·7		20·4	34·5		87·7

R_W was always greater for sugar-beet (Table 6). R_A was similar for both species until the last 2 intervals, when it became greater for potato because of the rapid development of side shoots. Sugar-beet had the smaller F after the third interval.

TABLE 6

Relative Growth-rate (R_W), Relative Leaf Growth-rate (R_A), and Leaf-area Ratio (F) of Sugar-beet, Potato, and Barley. Week 1 was 19 Days after Sowing Sugar-beet and 11 Days after Sowing Barley and Planting Potatoes

		R_W, per cent./week			R_A, per cent./week			F, cm.^{2}/g.		
Expt.	Week	Sugar-beet	Potato	Barley	Sugar-beet	Potato	Barley	Sugar-beet	Potato	Barley
2 and 4	1–2	212	150	153	207	202	191	182	111	299
,, ,, ,,	2–3	169	141	136	147	150	90	159	150	249
1	2·3–4·3	131	131	91	112	140	88	147	176	250
2 and 4	3–4	135	117	89	114	106	68	128	146	185
3	3–5	—	—	75	—	—	85	—	—	286
2	4–5	93	92	—	62	80	—	97	136	—
1	4·3–6·3	70	50	44	42	50	37	95	130	222
2	5–6	72	61	—	35	52	—	76	121	—
3	5–7	—	—	41	—	—	12	—	—	222
	Mean	126	106	90	103	111	82	126	139	245
1	S.E.	11·0	11·0	11·0	8·3	8·3	8·3	14·0	14·0	14·0
2 and 4		5·4	5·4	3·3	5·5	5·5	1·8	5·1	5·1	12·9
3				14·0			14·3			5·6
Mean		2·8	2·8	4·2	2·4	2·4	3·9	3·3	3·3	4·1

Experiments 3 and 4. Dry weight and shoot number per pot 25 days after sowing were almost identical in Expts. 3 and 4 and leaf area was slightly, though not significantly, lower in Expt. 4 (Fig. 1). One week later, at the last sampling of Expt. 4, the difference in leaf area was greater. The discrepancy between experiments cannot be ascribed to differences in mean temperature or light intensity, which were less than 0·5° C. and 50 f.c. Apart from this low leaf area 32 days from sowing there was good agreement between Expts. 1, 3, and 4.

E_A did not vary significantly between the 3 intervals of Expt. 4 (11–32 days from sowing) but fell during the next 4 weeks (Fig. 2, Expt. 3), confirming the decrease with age found in Expt. 1. During the last interval of Expt. 3 E_A increased, presumably because of assimilation in the ears that started to emerge 14 days before the final sampling. Over the period covered by all experiments, but excluding the final harvest of Expt. 3, E_A fell nearly linearly with time. The linear regression coefficient of E_A on time for all experiments was $-8{\cdot}7 \pm 1{\cdot}2$. E_N and E_W decreased approximately linearly to 78 and 64 per cent. respectively of their initial values in 3 weeks (Fig. 2). Only the decrease in E_W was significant. The linear regression coefficients were: E_N $-4{\cdot}12 \pm 2{\cdot}49$, E_W $-0{\cdot}466 \pm 0{\cdot}150$. E_A of barley was much smaller than that of sugar-beet or potato obtained in other experiments. The highest value for barley was similar to the smallest for potato and less than the smallest for sugar-beet. E_N and E_W of barley were similar to those of potato, and less than those of sugar-beet.

R_W, R_A, and to a smaller extent F, fell with age (Table 6). The average values for all barley experiments of R_W and R_A were less, and of F more, than those of sugar-beet and potato.

Discussion

Measurements in a controlled environment over periods ranging from 4 to 7 weeks during the early part of the growth period showed that mean relative growth-rate (R_W) and mean net assimilation rate based on leaf area (E_A) were greatest for sugar-beet, less for potato, and still less for barley, confirming the differences in E_A found between these crops in the field (Watson, 1947). Net assimilation rates based on leaf N (E_N) and leaf weight (E_W) were greater for sugar-beet than for the other 2 species. All these growth attributes decreased with time, i.e. with increasing age of the plant. The decrease in E_A was very slight for sugar-beet but considerable for the other 2 species. Relative leaf growth-rate (R_A) and leaf-area ratio (F) fell with time similarly for all species.

Before discussing the importance of differences in E_A it must be established that the usual method of calculation (p. 360) gave unbiased estimates of the mean rates of dry weight increase per unit leaf area. Williams (1946) showed that this is so only when the leaf area–dry weight curve is linear over the interval between samplings, and that E will be overestimated when the curve becomes concave to the dry-weight axis. Such a departure from linearity tends to develop later in the growth period and the resultant underestimation of E_A could mask a fall in E_A with time. However, such errors are usually small with intervals between samplings of less than 2 weeks. In the present experiments the interval between samplings was 1 or occasionally 2 weeks and the leaf-area/dry-weight relation was approximately linear. The biggest departure from linearity occurred with sugar-beet in Expt. 2. To ensure that this was not masking the true fall in E_A and thus exaggerating the difference between sugar-beet and potatoes, E_A for sugar-beet was calculated for daily intervals by the graphical method described by Williams (1946). In any weekly interval the largest difference between the mean of these daily values and E_A calculated from the initial and final mean dry weights and leaf areas was 7 per cent. and the average difference was only $2\frac{1}{2}$ per cent. Therefore there is no doubt that in these experiments E_A fell with age for potato and barley but not, or very slightly, for sugar-beet, and was greatest for sugar-beet and least for barley.

The difference between E_A of barley and that of the dicotyledonous crops is larger than previously reported because E_A of barley was based on leaf area including sheath as well as lamina. The proportion of the total area accounted for by sheath increased from 20 per cent. at the beginning of the experimental period to 46 per cent. at the end, so that neglecting the sheaths would have decreased the fall of E_A with time and lessened but not eliminated the difference between E_A of barley and that of the other 2 species. The proportion of total leaf dry weight and leaf N contributed by the sheaths varied little between sampling times and never exceeded 15 per cent., so E_N and E_W would have

been altered only slightly had the sheaths been neglected in Expt. 4. This would not be true of older plants. It is unfortunate that so much of the discussion about constancy of E is based on data for cereals in which leaf area was estimated as lamina area only. As the ratio of sheath to lamina increases with age, any fall in photosynthetic efficiency with age is obscured if the dry-matter production of the laminae and sheaths is attributed wholly to the laminae.

A decrease in the ratio of photosynthesizing to respiring tissue could contribute to the fall of E_A with time, provided the rate of respiration did not decrease much with age. The decrease with age in E_A of sugar-beet and barley could be accounted for by the concurrent fall in F, but the initial decrease in E_A of potato was accompanied by an increase in F. A difference in F between sugar-beet and potato did not contribute to the differences in E_A; in the first interval sugar-beet had the greater F but smaller E_A and in the last 3 intervals it had the smaller F but larger E_A.

A difference in the spatial arrangement of leaves between sugar-beet and potato may partly account for the greater mean E_A of sugar-beet as well as for its smaller change with time. The leaves of potato were distributed through a greater depth below the plane where light intensities were equalized than were those of sugar-beet, so that the average intensity of light falling on the leaves was greater for sugar-beet, especially towards the end of the experiment. The distances of the oldest leaves below the plane of equalized light intensity were, for sugar-beet and potato respectively, 2 and 3 cm. at sampling 1, and 18 and 23 cm. at sampling 6. Potato plants may also have received less light energy than sugar-beet because the upper (younger) leaves tended to shade the lower (older) leaves more in potato than in sugar-beet. This occurs because all potato leaves are approximately horizontal and have short petioles whereas the upper sugar-beet leaves are more upright and occupy the space between the longer petioles of the lower horizontally-held leaves.

Self-shading, particularly of the sheaths, probably occurred in barley in the later stages of growth and contributed to the decline in E. The maximum density was 70 shoots with a leaf area of 90 dm.^{2} in a pot whose surface area was only 3 dm.^{2}

Differences in gross morphology that lead to different degrees of shading may be more important in causing specific differences in E in artificial conditions where the maximum light intensity is probably insufficient to saturate the upper leaves, than in sunlight where even partially shaded leaves may have optimum illumination. Morphological differences resulting in differential shading of the lower leaves are unlikely to account entirely for the differences in E_A between the three species, especially as these occurred between young plants with less than 10 dm.^{2} leaf surface per pot, and internal factors must also be concerned.

Differences in E between species depend on when the measurements are made because E may fall as much as 15 per cent. of its initial value in a week. In the present experiments the period between sowing and equivalent sampling

times was 1 week longer for sugar-beet than for barley, but the conclusions are almost unchanged when sugar-beet and barley are compared at equal times from sowing. Even when the time for starting comparisons can be fixed satisfactorily, the results may depend on the proportion of the growth period of the different species covered, because the proportion of young to old leaves changes during the growth period and may affect E_A. If the photosynthetic activity of the leaves decreases as they become senescent, E_A of barley, which stops producing leaves when it flowers, will decrease with age more than that of sugar-beet, which remains vegetative and continues to produce new leaves. A low proportion of young to old leaves may have been a cause of the low E_A of barley at the end of the present experiments, but E_A of barley was also much less than that of sugar-beet earlier when both species were still vegetative. Leaves were produced on the main stem of sugar-beet and potato at similar rates although at the end of the experiment potato produced a terminal flower-bud, so the smaller E_A of potato was not a consequence of a smaller ratio of young to old leaves. When considering the relevance of specific differences in E_A to potential production by different crops, the value attainable when leaf-area index is high and when environmental factors favour maximum assimilation is important rather than the average over the whole growth period.

Differences in E between species may vary with the environment (Blackman and Wilson, 1951). The difference between sugar-beet and potato may have been influenced by the failure of the potato plants to produce tubers, probably because the daily period of light was too long or the night temperature too high. Rapid export of carbohydrate to a storage organ, as in the sugar-beet plants, might permit a higher rate of photosynthesis by decreasing carbohydrate content of the leaves. However, Watson (1947) found a similar difference in E between sugar-beet and potatoes that were forming tubers.

The use of other bases besides leaf area for expressing E has been discussed by Watson (1952). If the basis effectively measured the capacity of the system responsible for dry-matter accumulation E should vary with external factors but not with age or species. As E is primarily determined by the rate of photosynthesis, which is conventionally based on unit leaf area, E is also commonly expressed on the same basis. Williams (1946) claimed that leaf protein N is preferable, and that E calculated on this basis reflects changes in the environment more closely than E_A. Leaf weight is generally accepted to be a poor basis because E_W usually decreases rapidly with age, but it has frequently been used because it is easy to measure. Expts. 2 and 4 provide data for a comparison of E calculated on leaf area, leaf weight, and leaf nitrogen. As all the leaf material was dried at 80° C. it was unsuitable for the determination of protein N, but protein N would be approximately proportional to total N for leaves of this limited range of ages, within if not necessarily between species. Unfortunately, E_N and E_W for barley were only determined for the first 3 weekly intervals, as only in Expt. 4 was the sheath separated from the underlying stem before determining its dry weight and nitrogen content.

Taking constancy of E with age as a criterion, these results show that leaf N is no better basis than leaf area because E_N never fell less with time than E_A, and for sugar-beet it fell more. The rates at which E_A, E_W, and E_N decreased during the experimental period can be compared by expressing the regression coefficients of E on time as percentages of E during the first interval (Table 7).

TABLE 7

Linear Regression Coefficients of E_A, E_N, and E_W on Time, expressed as per cent. per Week of Value for Interval between Weeks 1 and 2 (see Fig. 2)

	E_A	E_N	E_W
Sugar-beet (Expt. 2)	$-4{\cdot}0\pm2{\cdot}2$	$-11{\cdot}4\pm1{\cdot}4$	$-12{\cdot}6\pm1{\cdot}3$
Potato (Expt. 2)	$-15{\cdot}0\pm1{\cdot}6$	$-14{\cdot}1\pm1{\cdot}5$	$-16{\cdot}1\pm1{\cdot}4$
Barley (Expt. 4)	$-2{\cdot}7\pm7{\cdot}6$	$-10{\cdot}6\pm6{\cdot}4$	$-17{\cdot}4\pm5{\cdot}6$
Barley (Expts. 1, 3, 4)	$-16{\cdot}8\pm2{\cdot}4$	—	—

Except for the small or negligible effect of time on E_A of sugar-beet and on barley during the early part of the growth period, all expressions of E fall at similar rates of approximately 14 per cent. per week. The regression for barley is not a good estimate of the rate of decrease of E_A with time because it is derived from the results of 3 separate experiments which may differ in other factors besides age of plants. E_A at the beginning of Expt. 3 was low compared with that at the end of Expt. 4 because of the unexplained difference in leaf area (p. 366), and E_A for all species in Expt. 1 tended to be low, perhaps because of lower light intensity, at least compared with Expt. 2 (p. 359). E_A fell slightly less than E_W with age because of the usual tendency for leaf weight per unit area to increase in older plants. This tendency and the resulting difference in time trend of E_A and E_W was very small in potato. Nitrogen per cent. of dry weight in leaves usually decreases with age and such a tendency caused E_N to decrease slightly less than E_W. The relationship between E_A and E_N depends on the relative size of changes with age in dry weight per unit area and in N per cent. of dry weight. As these differed between species there was no uniformity in the relative time trends of E_A, E_W and E_N and none of them was constant for any considerable period, even in young actively-growing material. Williams (1946) found that E_N fell more rapidly with age in plants with a high than a low N status, and supposed that this happened because storage protein accumulated, so that leaf protein N was no longer a good measure of the metabolically active cytoplasm. Whether or not this explanation is correct, the results of the present experiment agree with the conclusion that E_N decreases with age for plants that have a high N content. Nitrogen per cent. of dry matter of the leaves ranged from 5·3 to 7·5, but this would be expected in well-nourished young plants, and it was not a consequence of a deficiency of other nutrients, e.g. phosphorus, as in some of the experiments discussed by Williams. There seems to be little point in using leaf protein N as a basis for expressing E, if its advantages are restricted to a narrow range of low or unbalanced N supply.

These experiments, done in a controlled environment, support the conclusion reached earlier by Watson (1952) that leaf area is the most suitable basis for expressing net assimilation rate, although it does not wholly satisfy the criterion that E_A should be independent of age and species. Some of the variation of E_A in a constant environment may be caused by different plants affecting their microclimate differently, e.g. in the degree of self-shading; by change in the proportion of photosynthesizing to respiring tissue; or by differences in internal factors between plants that make area an inadequate measure of the photosynthetic capacity of the leaves. Which of these causes operates in any particular instance can be discovered by further analysis.

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Observations on Net Assimilation Rates in Arctic Environments[1]

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With two Figures in the Text

Abstract

Examination of the net assimilation rate (E) during the growing season in arctic regions by a detached-leaf method revealed no differences between species or with soil richness, but showed a reduction of E with exposure to wind—probably resulting from cooling—and a tendency for E to fall towards the later part of the growing season. E generally lay in the range 0·5 to 0·8 g./dm.^{2}/week.

E for detached leaves ignores respiratory losses in other parts of the plant and is not comparable with E for whole plants; failure to appreciate this confused a previous comparison of E under arctic and temperate environments. E for detached leaves in temperate summer conditions is normally around 1·1 to 1·5 g./dm.^{2}/week. Thus E is reduced in arctic environments to about half the value in temperate conditions. This reduction is due mainly to the cold climate.

Introduction

THE only recorded net assimilation rate, E (rate of increase of dry matter per unit leaf area), for plants under field conditions in an arctic environment is that of Russell (1940) for *Oxyria digyna* at Jan Mayen Island in the Greenland Sea. Russell measured the 'carbohydrate' content (sugars+starch) of detached leaves before and after a period of 6 days standing in the open with petioles in wet sand. On the assumption that the increase in weight of 'carbohydrate' equalled the increase in dry matter, his experiment gave $E = 0{\cdot}30$ g./dm.^{2}/week. For several reasons Russell regarded this value as an underestimate; and although he suggested that 'the net assimilation rates of arctic plants are at least comparable with those of plants in temperate regions', he emphasized that this single result was not a sufficient basis for generalization. He had, however, demonstrated a method of estimating E that is especially convenient for field work under arctic conditions, and this paper describes further investigations based on a similar method.

Methods

These investigations were made mainly at Jan Mayen Island (71° N.), and also at Cornwallis Island in the Canadian Arctic (75° N.). These islands fall within the 'middle-arctic' and 'high-arctic' zones of Polunin (1951) respectively; summer temperatures are around 5° C. (Table 1), and the vegetation

[1] Botanical results No. 7 of the Oxford University Expedition to Jan Mayen Island (1947), the Jan Mayen Expedition (1950), and the Cornwallis Island Expedition (1954).

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consists typically of scattered dwarf perennial flowering plants, some bryophytes and lichens, and much bare ground.

TABLE 1

Monthly Averages of Daily Temperatures (°C.)

	Jan Mayen Island		Cornwallis Island	
	Min.	Max.	Min.	Max.
June	1·4	5·9	−1·9	2·8
July	4·0	8·0	1·6	7·3
August	4·9	8·8	0·4	5·3
September	2·3	6·2	−6·9	−2·6

For each experiment, mature and healthy leaves (generally of *Oxyria digyna*) were gathered, and the petioles were cut off to leave a stump about 1 cm. long. Two comparable groups of 25 leaves were then taken:

(i) one was weighed, dried on a boiling-water bath, and reweighed;
(ii) the other was set with the petiolar stumps in sand that was kept wet, exposed for 1 to 3 days in the open, and then weighed before and after drying.

A further sample of leaves was used for determining leaf areas. Each such experiment was replicated, usually sixfold, and values of E are expressed below as means with standard errors.

This method makes certain assumptions:

1. It is assumed that leaf areas remained constant during each experiment. The selected leaves were mature, and even seedling leaves have low rates of expansion in the arctic environment (1–2 per cent. per day at Cornwallis Island).

2. It is assumed that the products of assimilation were not translocated away from the leaves. Several investigations have shown that only insignificant amounts escape through the cut petioles of detached leaves standing in water (Yarwood, 1946).

3. It is assumed that the assimilation rate of the leaves was not affected by detachment. This point has been examined most carefully by Bauer (1935). Although detachment inevitably initiates various physiological changes, Bauer finds that assimilation rates are not affected over short periods. Over longer periods, however, assimilation rates fall as a result especially of increasing carbohydrate and decreasing water concentrations (Yarwood, 1946). Thus 9 days after detachment E for leaves of *Oxyria digyna* at Jan Mayen Island was about half of E for newly detached leaves; by this time the leaves were slightly yellowed, they had doubled their dry weight, and their moisture content had fallen from 86 to 82 per cent. in spite of some uptake of water. Accordingly, observations at Jan Mayen Island were based on a 3-day period, during which the falling off in calculated E probably amounted to only a few per cent. (Fig. 1). For *Salix arctica* at Cornwallis Island, where the

climate is less moist, E fell more rapidly; the results quoted below are therefore based on a 1-day period.

In three experiments in which for some unknown reason water was lost from the detached leaves, E averaged only some two-thirds of the normal value, and these results have been rejected. Thoday (1910) records a similar depression of E with loss of turgidity in detached leaves of *Helianthus annuus* in England. Indeed, under temperate summer conditions the method

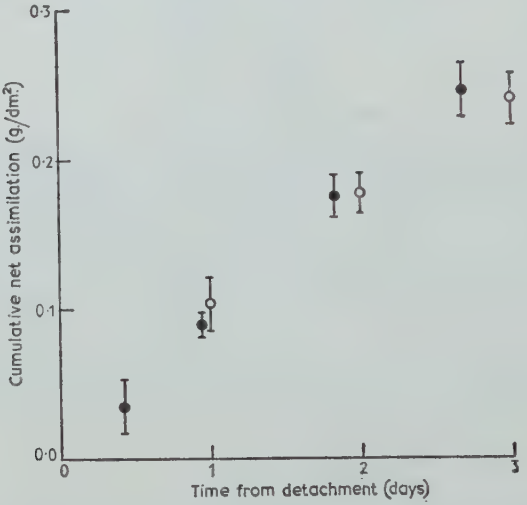

FIG. 1. Progress of net assimilation during the first 3 days after detachment of leaves. Closed circles, *Oxyria digyna* at Jan Mayen Island; open circles, *Salix arctica* at Cornwallis Island. Vertical lines represent standard errors. Data averaged from experiments commenced on 3 different days (for *Oxyria*) and 10 different days (for *Salix*).

described here cannot be used, since E falls off rapidly in leaves exposed in the open. It is only the low temperatures of arctic regions, coupled, at Jan Mayen Island, with exceptionally moist conditions, that allow detached leaves to continue assimilating at virtually normal rates for a day or more, and so permit observations of the type described here.

VARIATION BETWEEN SPECIES

Leaves of three species growing together in a single community at Jan Mayen Island were collected on 24 August for a comparison of E between species. There are rather marked differences between the leaves of these species in shape, colour, and fleshiness as well as in the features described quantitatively in the last three columns of Table 2. Nevertheless, E was remarkably uniform throughout.

It has been found in temperate regions that differences in E between dicotyledonous species, although statistically significant, are small (Watson, 1947). Accordingly, the uniformity of estimates of E in Table 2 is not sur-

prising; plainly, however, more accurate determinations for a greater number of species might well reveal differences.

TABLE 2

Net Assimilation Rates of Three Species, together with Certain Characteristics of their Leaves

	E (g./dm.²/week)	Leaf area (cm.²/leaf)	Leaf area / Leaf weight (dm.²/g.)	Moisture content (% fr. wt.)
Oxyria digyna . . .	0·54±0·08	5·07	1·71	87·4
Salix herbacea . . .	0·53±0·07	1·06	1·05	66·7
Sibbaldia procumbens . .	0·50±0·05	1·81	1·12	63·0

VARIATION WITH HABITAT

Two types of observation on variation in *E* with habitat were made, both using *Oxyria*:

(i) A comparison was made of *E* for leaves that had been gathered from four different habitats but were then exposed in a single environment; any variation found would be due to internal differences in the leaves resulting from edaphic differences between their original habitats, or, less probably, from climatic differences or ecotypic differentiation.

(ii) A comparison was made of *E* for leaves that had been gathered from a single habitat but were then exposed in two contrasting environments; any difference in *E* would in this case be due to climatic differences between these two environments.

For observation (i) leaves were collected on 12 August from the following sites:

Desert: open vegetation on a south-facing bank of poor, sandy soil.

Austrian Station: pure stand of *Oxyria* in an otherwise barren sandy valley where the soil was locally enriched by human habitation during the Polar Year of 1882–3.

North Lagoon: fairly rich sward of *Oxyria* in a sheltered situation beside a lagoon.

Goose Pasture: closed sward of flowering plants on rich soil on a sheltered, south-facing bank; occasional grazing and manuring by geese.

'Desert' and 'Goose Pasture' represent almost the extremes of poverty and luxuriance of ecosystems at Jan Mayen Island. The great difference between these sites is indicated by soil analyses and by leaf areas (Table 3). However, there was no indication of variation in *E* between habitats ($F = 0{\cdot}77$) or of a trend in *E* with habitat richness.

For observation (ii) comparable sets of leaves were simultaneously exposed on 25 August on a windy hilltop and in a sheltered situation on the hillside below. Table 4 shows that the corresponding variations in *E* were proportionately greater than those associated with either species (Table 2) or source

habitat (Table 3). A direct comparison of final leaf weights in the two climates shows that the difference is statistically significant ($t = 3{\cdot}87$; $P < 0{\cdot}02$).

TABLE 3

Net Assimilation Rates in a Uniform Environment of Oxyria digyna *Leaves from Four Different Sites, together with Data on Leaf Areas and Soil Characteristics*

	E (g./dm.²/week)	Leaf area (cm.²/leaf)	Soil moisture (% fr. wt.)	Soil nitrogen (% dry wt.)
Desert	0·64±0·05	1·3±0·1	6·4	0·020
Austrian Station	0·61±0·05	5·8±0·2	12·6	0·022
North Lagoon	0·56±0·05	7·1±0·3	16·1	0·076
Goose Pasture	0·66±0·05	6·4±0·3	38·3	0·341

TABLE 4

Net Assimilation Rates in Two Different Climates of Oxyria digyna *Leaves Gathered from a Single Source*

	E (g./dm.²/week)
Windy hilltop	0·34±0·02
Sheltered hillside	0·46±0·05

It is probable that the climatic factor responsible for this difference in E was temperature. Light intensity can be excluded since differences in it were slight and such as to favour the exposed leaves. Water status seems not to have been responsible, for although the windswept leaves took up less water during the experiment than the sheltered leaves their final moisture content (83·5 per cent.) was as high as that of the sheltered leaves (83·1 per cent.) owing to their smaller dry weight increase. On the other hand, air temperatures were lower at the exposed site (measurements around noon on four days showed differences of 1·4°, 2·0°, 1·7°, and 0·8° C.), and in addition the stronger wind will have caused greater cooling of leaves during sunny weather (Warren Wilson, 1959). Moreover, a reduction of E by temperature is in accord with conclusions reached below.

Variation with Time of Year

When all the comparable estimates of E are plotted against the time of year when they were made, there is an indication of a progressive fall in E from 0·8–0·9 g./dm.²/week at the end of June to 0·4–0·5 g./dm.²/week at the end of August. This is expressed by the linear regression fitted to the 62 estimates that are available for the period 29 June–27 August:

$$E = 0{\cdot}87 - 0{\cdot}045X,$$

where X is the time in weeks after 29 June (Fig. 2). The regression coefficient indicates that E was significantly decreasing ($t = 3{\cdot}40$; $P < 0{\cdot}01$).

This decrease in E resembles the trend that occurs at the same season in temperate regions, where it is a result mainly of decreasing day-length, light intensity, and temperature (Watson, 1958).

The period covered by the observations at Jan Mayen Island represents the central part of the growing season; there are probably a few weeks of activity before and after this period. The climate is such that E for the first few weeks of the season is unlikely to exceed the values observed in late June, while during September E will continue the falling trend observed during August.

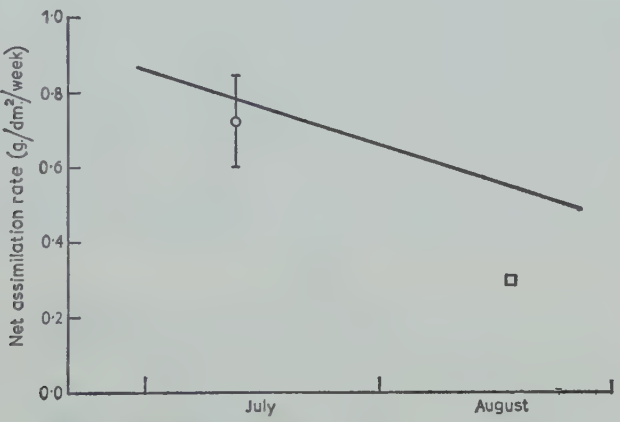

Fig. 2. The sloping line shows the regression of net assimilation rate (E) on time of year at Jan Mayen Island during July and August. The circle indicates an estimate of E for *Salix arctica* at Cornwallis Island. The square shows the value of E obtained by Russell (1940) for *Oxyria digyna* at Jan Mayen Island.

Other Data on Arctic Net Assimilation Rates

A further estimate of E under arctic conditions is available for detached leaves of *Salix arctica* exposed in ten experiments between 6 and 17 July at Cornwallis Island. This value ($0{\cdot}73 \pm 0{\cdot}12$ g./dm.²/week) is plotted in Fig. 2; it is of the same order as E for detached leaves of *Oxyria digyna* at Jan Mayen Island.

Russell's estimate of E of 0·30 g./dm.²/week for detached leaves of *Oxyria* exposed at Jan Mayen Island over the period 15–20 August is only a little over half the values obtained for the same time of year in the present work (Fig. 2). This discrepancy must be due at least in part to the longer period (6 days) for which Russell's leaves were exposed; this will have led, as he pointed out, to depression of E.

Wager (1941), working at 69° N. in East Greenland, measured by gas analysis the rates of apparent assimilation of *Oxyria digyna* leaves in an assimilation chamber at various intensities of daylight and at controlled temperatures of 0° and 10° C. Although his observations were not designed

to assess rates of dry-matter accumulation in the natural arctic environment, his results can be turned to this purpose by relating them to the light intensities recorded at Jan Mayen Island during July and August by Russell and Wellington (1940). If the equivalence of carbon dioxide to dry matter is taken to be in the ratio 1 : 0·65, values of E at 0° and 10° average 0·68 g./dm.^{2}/ week. In view of the various assumptions made in arriving at this figure, no great weight should be given to it; but in fact it agrees with the estimates of E recorded above.

In attempting to draw any general conclusion about the level of E in arctic environments it must be recognized that:

(i) the available data are narrowly based; values for other species and other arctic lands are needed;

(ii) E for detached leaves placed on sand is likely to differ from E for leaves occurring naturally in swards, as a result of microclimatic differences (especially in light intensity).

With these reservations, it seems from all the available information presented above that E for detached leaves exposed to normal, summer arctic conditions is of the order of 0·5 to 0·8 g./dm.^{2}/week.

Comparison of Arctic and Temperate Net Assimilation Rates

In 1938 Heath and Gregory examined all available estimates of E and concluded that 'there is extraordinarily little variation in the mean net assimilation rate for the early part of the life cycle for a number of very different species in the most diverse environments'; the estimates lay between 0·41 and 0·72, with a mean of 0·55 g./dm.^{2}/week. Accordingly, when Russell in 1940 estimated E for detached leaves at Jan Mayen Island to be 0·30 g./dm.^{2}/ week (and he considered this an underestimate) he tentatively suggested that E in arctic regions was comparable with E in temperate regions. The mean values found in the present work, varying between 0·34 and 0·86 g./dm.^{2}/week, seem to support Russell's suggestion in being closely comparable with the values quoted by Heath and Gregory for temperate plants.

However, this comparison is not legitimate. The values of E quoted by Heath and Gregory for temperate regions are based on whole plants, whereas values of E for arctic regions are based on detached leaves. The latter values are therefore enhanced by the net respiratory losses in all organs other than the leaves. These losses are considerable, particularly in arctic species since these have especially rapid respiration (Wager, 1941). The respiration rate of detached leaves of *Oxyria* at Jan Mayen Island in early August, estimated by finding the loss in dry weight of leaves kept on moist sand in the dark over a 5-day period, was 0·11 g./dm.^{2}/week. This agrees with Wager's data on respiration rates of *Oxyria* leaves at controlled temperatures in Greenland: 0·06 g./dm.^{2}/week at 0° C., and 0·19 g./dm.^{2}/week at 10° C. Thus the respiration rate of the *Oxyria* leaves is equal to about one-sixth of their net

assimilation rate. Other organs will generally respire more slowly, but even so they will cause considerable loss of assimilate, for they represent some 85 per cent. (dry weight) of the plant. Since, therefore, E for detached leaves must exceed E for whole plants by a considerable amount, the estimate of E for detached leaves in arctic conditions must be compared with values of E for detached leaves, not whole plants, in temperate conditions.

It has been pointed out already that under temperate summer conditions detached leaves lose water so readily that they cannot be used for estimating E over a period of a day or some days. However, Sachs (1884) showed that it is possible to employ them for a period of some hours, and hence to calculate E if certain assumptions are made about day-length and respiration rate; alternatively, various half-leaf methods give comparable estimates. Table 5 shows E for leaves of various species under temperate conditions; for detached-leaf experiments a day-length of 12 hours has been assumed (Sachs assumed a 15-hour day, but Broocks and Miller have shown that rates of dry-weight increase do not reach daytime level until 5 or 6 a.m.); and a respiration rate of 0·15 g./dm.²/week has been assumed, this being the mean at 20° C. for leaves of 26 herbaceous species of temperate lands quoted by Stocker (1935).

TABLE 5

Mean Net Assimilation Rates for Leaves of Various Species in Temperate Conditions

Author	Location	Time of year	Species	Method	E (g./dm.²/week)
Sachs (1884)	Würzburg (49°)*	August	*Helianthus annuus*	Detached leaf	1·38
,,	,,	,,	,, ,,	Detached half-leaf	1·31
,,	,,	July	,, ,,	Half-leaf	1·51
,,	,,	,,	*Cucurbita pepo*	,,	1·19
Broocks (1892)	Halle (52°)	Aug.–Sept.	*Beta vulgaris*	,,	0·64
Thoday (1910)	Cambridge (52°)	July–Aug.	*Helianthus annuus*	Detached half-leaf	1·35
Miller (1917)	Garden City (37°)	,,	*Zea mays*	Half-leaf	0·91
,,	,,	,,	*Andropogon sorghum* (kafir)	,,	0·82
,,	,,	,,	*Andropogon sorghum* (milo)	,,	1·10

* Degrees of latitude.

It should be noted that the lower values of E in Table 5 are depressed for various special reasons below normal levels. The plants examined by Miller suffered from severe water deficiency—they often wilted—and this halved the rate of dry-weight gain after 3 p.m. in milo, and after about 10 a.m. in corn and kafir. In the sugar-beet studied by Broocks dry-weight gain ceased around noon, presumably again because of water shortage; moreover, his observations were made towards the end of the growing season.

Thus it seems probable that under normal summer conditions E for leaves in temperate environments lies between about 1·1 and 1·5 g./dm.²/week. If this is so, it is roughly twice as great as E for leaves in arctic environments.

This conclusion is of interest in connexion with the proposition of Heath and Gregory (1938) restated by Gregory (1950) that E 'varies little with the latitude at which the vegetation grows'. This statement has not always been

accepted, but the only published data bearing on it are those of Blackman and Black (1959), who find that values of E for plants in subtropical and tropical regions 'greatly exceed those so far recorded for cool temperate regions' as a result, they conclude, of the difference in light receipt. The present work suggests that E in arctic regions is as far below E in temperate regions as E in tropical regions is above; but it is probable that light receipt is not in this case the factor responsible. Indeed, the mean daily radiation from sun and sky during July is greater at Cornwallis Island than in England:

Resolute, Cornwallis Island	510 g.cal./cm.^{2}/day
Kew, England	350 g.cal./cm.^{2}/day.

Detailed discussion of the control of assimilation rates under arctic conditions will be deferred to a later paper on the growth analysis of whole plants, but there can be little doubt that it is the low temperatures prevailing even during the summer that are basically responsible for the depression of E in arctic environments.

Acknowledgements

This field work was made possible by assistance of various kinds generously given by the many individuals and organizations to whom detailed acknowledgement was made in the fourth paper in this series. I should like to record here again my gratitude to them.

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Movements of Organic Nitrogen and Carbohydrates in *Pelargonium* Plants

BY

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Abstract

Evidence has been obtained suggesting that the movement of organic nitrogen in the phloem is independent of carbohydrate movement in *Pelargonium* plants. The results obtained cannot readily be explained by the mass-flow theory of Munch.

Introduction

THE mass-flow hypothesis proposed by Munch (1930) as the mechanism for translocation implies that all compounds present in the sieve-tubes should move together; the direction of movement being presumably related to that of the carbohydrates. Such movement has been claimed for the movement of virus molecules and growth regulators (Crafts, 1951). Other theories for the mechanism of translocation do not imply this, and compounds present in the sieve-tubes might be considered to move independently of each other. Thus the demonstration of independent movement in the phloem would therefore give valuable experimental support for these latter theories.

Experiments designed to show independent movement of organic compounds include those of Phillis and Mason (1936); Mason, Maskell, and Phillis (1936); Fischer (1936); and Palmquist (1938). More recently, Chen (1951) followed the simultaneous movement of carbohydrates labelled with isotopic ^{14}C and phosphate labelled with ^{32}P and concluded that simultaneous movement in opposite directions in the sieve-tubes was possible. A similar conclusion was reached by Bonner (1944), who followed the rate of accumulation of several organic compounds above rings cut in the lower stem region in tomato plants.

The earlier experiments of Mason and Maskell (1928) demonstrated that the translocation of carbohydrates in the phloem of cotton plants was determined by the prevailing concentration gradients for sucrose in the sieve-tube region. If the direction of movement of organic nitrogen is also determined by concentration gradients in some mobile nitrogen fraction, then the movement of nitrogen might be expected to be independent of that of carbohydrate. It has recently been shown (Turner, 1960) that the accumulation of organic nitrogen compounds does not occur or is much reduced in amount above rings cut in the lower stem region of *Pelargonium* plants when the growing apices were left intact. Thus the rate of accumulation of organic nitrogen in the stem after ringing could be varied by the experimental treatment. In the

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experiments described below, the movement of carbohydrate was also followed under these conditions and compared with the movement of organic nitrogen to determine whether independent movement of organic carbon and nitrogen is possible in *Pelargonium* plants.

Materials and Methods

Pelargonium plants grown from cuttings were used. The plants were kept in a greenhouse at 25° C. before and during the experiments. Extracts were made and the determinations of the concentration of the various nitrogen fractions were carried out as described in the previous paper (Turner, 1960).

The methods used for the estimation of reducing sugars and sucrose were those of Narain (1932) after the extracts had been cleared as described by El Gawadi (1935). The extraction and estimation of starch was carried out using the method of Hanes (1936). The β-amylase preparation required for this method was prepared from potato tubers and was assumed to give 60 per cent. conversion of the starch to maltose.

Results

Six plants were used, each with a bifurcating stem. These stems were allotted to three groups designated (1) initial control, (2) ringed; apices of shoots left intact, (3) ringed apices removed. It was arranged that the stems on the same plant were not allocated to the same group.

On the day of ringing a portion of phloem tissue was removed in the initial control group sample for total nitrogen determination from a position corresponding to the position of the ring in the two ringed groups. In addition, phloem tissue was taken from immediately above the region sampled for total nitrogen content and divided into (*a*) phloem and cortex, and (*b*) remainder of the stem, which for convenience will be termed 'xylem' but will in *Pelargonium* plants also consist of pith tissue. The material collected from the plants in each group was pooled into one sample each of phloem and xylem tissue, and extracted for soluble nitrogen and carbohydrate fractions.

In the ringed groups the phloem tissue actually removed by the ringing operation was used for total nitrogen determination, thus giving a value for each stem. At the time of ringing in one group of plants the growing apices of the stems were removed; in the other ringed group they were left intact.

Six days after ringing a sample of phloem tissue was taken from immediately above the ringed regions from each of the ringed stems for total nitrogen determination. As a value for the total nitrogen content was obtained for each stem before ringing, direct demonstration of the accumulation of nitrogen above the ring was possible. Samples of phloem and xylem were also taken as described for the initial control group and extracted for soluble nitrogen compounds and carbohydrates. The results are shown in Table 1.

These results confirm the previous observations that the accumulation of nitrogen in the phloem above rings is reduced in amount when the growing stem apices are left intact and that the increase in total nitrogen on ringing can

be attributed to increases in the soluble nitrogen fractions. The results also demonstrate an accumulation of carbohydrates in the stem after ringing; the large increases in sucrose suggest that this compound is probably translocated in *Pelargonium*. The increased concentration of both soluble nitrogen and sucrose in the xylem after ringing indicates that a ready lateral transfer of organic material may occur between the xylem and phloem tissues. Microscopic examination of transverse sections of the stems in both control and ringed samples confirmed that there had been no apparent change in the starch content on ringing. This suggests therefore that the apparent accumulation of soluble carbohydrates in the stem after ringing cannot be due to hydrolysis of starch stored in the near-by pith tissue but must be due to the movement of carbohydrate into the region above the rings.

TABLE 1

Movement of Nitrogen and Carbohydrate in Pelargonium *Plants*

Nitrogen mg. N *per g. fresh weight*

	Total nitrogen		Total soluble N	
			Phloem	Xylem
Initial controls	1·01 1·00		0·24	0·60
	initial	final 6 days		
Ringed samples, apices removed 4 branches . .	0·87 0·94 0·95 1·13	1·67 1·98 2·13 1·45	1·03	1·40
	Av. 0·97	1·73		
Ringed samples, apices intact	1·19 0·91 1·02 0·93	1·50 1·24 1·60 1·02		
	Av. 1·01	1·34	0·63	1·10

Carbohydrate mg. per g. fresh weight

	Reducing sugars		Sucrose		Starch	
	Phloem	Xylem	Phloem	Xylem	Phloem	Xylem
Initial control . . .	1·96	3·27	2·00	0·20	3·27	6·62
Ringed, apices removed .	1·85	3·00	3·56	3·90	3·50	6·80
Ringed, apices intact . .	1·45	3·30	3·13	3·60	3·20	6·50

The total increase in concentration of sucrose in xylem and phloem tissues in the ringed plants (growing points removed) was 5·26 mg. The corresponding increases in soluble nitrogen are 0·89 and 1·59 mg. per g. fresh weight of material extracted. Thus the removal of the growing points has led to a decrease in the rate of accumulation of nitrogen compared with carbohydrate accumulation.

In further experiments an even greater independence of nitrogen movement compared with that of carbohydrate was recorded.

For example, in an experiment similar to that described above except that the final samples were taken 3 days after ringing, the increases in soluble nitrogen and carbohydrate fractions were as follows:

	mg. per g. fresh weight	
	Sol. N	Sucrose
Ringed, apices left intact	+0·10	1·20
Ringed, apices removed at time of ringing .	+1·05	0·90

In the plants with the growing apices left intact an accumulation of sucrose occurred without any significant accumulation of nitrogen.

Discussion

The experimental results indicate that when the apices are left intact in ringed *Pelargonium* plants, the rate of accumulation of sucrose above rings cut in the lower stem region is little affected compared with plants in which the apices were removed at the time of ringing. However, the accumulation of soluble nitrogen compounds is markedly affected since the accumulation is much reduced when the apices are left intact. This suggests that the export of organic nitrogen from the leaves occurs principally towards the developing apices, and that under these conditions little movement of organic nitrogen occurs towards the lower stem regions.

These experiments suggest that in *Pelargonium* the movement of nitrogen is independent of the movement of carbohydrates. This observation, together with the evidence obtained earlier that not all of the soluble nitrogen fractions in the phloem accumulation in the stem after ringing or accumulated at different rates, suggests that the movement of nitrogen in *Pelargonium* plants cannot be explained by the mass-flow theory unless additional hypotheses or modifications are made.

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The Movement of Organic Nitrogen Compounds in Plants

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With two Figures in the Text

Abstract

The hypothesis that the movement of organic nitrogen, like that of carbohydrate, is governed by the prevailing concentration gradients for the mobile compounds in the phloem has been tested by determining the effect on nitrogen distribution when normal translocation was interrupted by ringing in *Pelargonium* spp. and *Vicia faba* plants.

Nitrogen compounds in the plant tissue were fractionated into protein, soluble protein, amide, amino-acid, peptide, nitrate, and ammonia nitrogen. The presence of short-chain peptides in the extracts was confirmed by paper chromatography.

Results showed that accumulation above the ringed zone was confined to the soluble nitrogen fractions and that amino-acids and possibly peptides were involved in translocation. The direction of movement may be determined by the prevailing concentration gradients of these compounds in the phloem tissue.

Introduction

THE identity of the compounds translocated in the phloem tissue of plants and the mechanism involved has been the subject of much controversy.

Mason and Maskell (1928*a*, *b*) concluded that sucrose was the main form in which carbohydrates were translocated in the cotton plant and that the rate and direction of movement were governed by the prevailing concentration of sucrose in the sieve-tube region. They conceded, however, that movement by diffusion alone could not account for the measured rate of translocation.

In later experiments (Maskell and Mason 1929*a*, *b*; 1930*a*, *b*) the movement of nitrogen was also followed in cotton plants; but they were unable to draw any definite conclusions regarding the form in which organic nitrogen was translocated. Assuming that the downward movement of nitrogen in the bark, as demonstrated by ringing experiments, was also governed by concentration gradients, the only positive gradient which could account for the movement of the nitrogen was shown by a fraction termed 'residual nitrogen'. This fraction would include any peptide nitrogen, and non-amino nitrogen of the amino-acids present.

The importance of the residual nitrogen fraction in translocation has also been stressed by Street, Kenyon, and Watson (1946). However, other workers, Gregory and Sen (1937) and Chibnall (1939), have considered that amides

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might be translocated whilst Fowden (1954) and Bollard (1953) have indicated that other amino-acids may also be important. The presence of amino-acids in phloem sap has been reported by Mittler (1953). Petrie and Wood (1938) have further suggested that all the crystalloid organic nitrogen fractions are capable of being translocated.

On the other hand Leonard (1938) and Engard (1939) failed to demonstrate the accumulation of organic nitrogen above rings cut in the lower stem and concluded that inorganic nitrogen may be moved in the phloem.

The form in which nitrogen is translocated in the phloem tissue therefore remains obscure. In the experiments described below, use has been made of the chromatographic technique to identify more precisely the various nitrogen fractions which accumulate in the stems after ringing.

Materials and Methods

Either *Pelargonium* plants grown from cuttings or *Vicia faba* plants grown from seed in boxes were used. The plants were kept in a greenhouse at 25° C. before and during the experiment.

Total nitrogen and total soluble nitrogen was determined by digestion of the plant material or on aliquots of the extracts with conc. sulphuric acid and selenium oxide catalyst mixture, followed by distillation in a Markham still.

Soluble-nitrogen fractions were extracted by immersing the plant tissues in boiling ethanol for 3 minutes. The liquid was decanted off and the tissue pressed in a hydraulic press. The residue was again extracted with warm ethanol followed by a water extraction. The liquids were decanted off and combined into one sample. Aliquots of this sample were taken for total soluble-nitrogen content; for total amide determination (Chibnall and Westall, 1932), amino-acid concentration (Sorbel, Horschman, and Besman, 1945), and for nitrate and ammonia content (Schlenker, 1932). Two-dimensional chromatograms for amino-acids of the extracts were also run, using phenol-ammonia and butanol-acetic-water (4/1/5 v/v) as developing solvents and spraying with 0·1 per cent. ninhydrin in water-saturated butanol.

The difference in amount between total amino-acid content and total amide was termed 'free amino-acids'. Water-soluble protein nitrogen was determined by adding ethyl alcohol to the extracts to give a final concentration of 80 per cent. alcohol. The extract was kept at 0° C. for 3–4 hours, when a gelatinous precipitate was formed. This precipitate was filtered off and the soluble-nitrogen content determined on the filtrate. The difference between this value and that obtained before precipitation was attributed to the soluble-protein fraction.

The difference between the concentration of total soluble nitrogen and the sum of the water-souble protein nitrogen plus amide nitrogen plus amino-acid nitrogen plus any free ammonia nitrogen present was ascribed to a fraction termed peptide 'nitrogen'. The presence of peptides in the extracts was confirmed by acid hydrolysis of the extracts (Conc. HCl at 100° C. for 20 hours) after removal of the water-soluble protein fraction. Determination of

the amino-acid concentration showed an increase after the acid hydrolysis. This increase in amino-acid nitrogen could also be demonstrated by paper chromatography and shown to be due to increases in glutamic and aspartic acids, alanine, histidine, proline, valine, and leucine.

Results

Experiments with Pelargonium *plants*

With *Pelargonium* plants, ringing can be accomplished by cutting into the stem and removing a girdle of tissue external to the xylem. The material removed by the ringing operation will be referred to as 'phloem tissue'.

Direct demonstration of the accumulation of nitrogen compounds in the phloem tissue above rings cut in the stem is possible since the initial values for the total nitrogen content in the stem phloem can be determined on the

Table 1

Accumulation of Nitrogen above Rings Cut in the Stem (as mg. total N *per g. fresh weight of phloem tissue)*

	Initial	5 days after ringing
Branch 1 . . .	0·86	1·47
Branch 2 . . .	0·81	1·56
Branch 3 . . .	0·79	1·22

material actually removed by the ringing operation. This value can be compared with that obtained for similar material taken at the completion of the experiment; this sample being taken from immediately above the site of original ring.

Thus in Expt. 1 (Table 1) three branches on the same *Pelargonium* plant were ringed at their junction with the main stem. At the commencement of the experiment the growing apices and inflorescences were removed and the leaves darkened on the ringed branches. Five days after ringing samples of phloem tissue were taken from immediately above the original rings and the total nitrogen content determined.

No accumulation of organic nitrogen occurred in the phloem region below the rings.

It may be argued that as the initial and final samples have not been taken from the same position along the stem (i.e. the final sample being taken about 1 cm. nearer the apex of the branch than the initial sample), the apparent increase in nitrogen after ringing might be explained as being due to a possible steep concentration gradient existing in the phloem. That this is not so is shown by removing material from two rings spaced 1 cm. apart. The difference in total nitrogen between these two regions was found to be 0·07 mg.

Having demonstrated the accumulation of nitrogen in the stem after ringing, attempts were made to identify the nitrogen fractions responsible for this accumulation. Phloem tissue was sampled from above the rings and extracted for soluble-nitrogen compounds. Phloem taken from corresponding positions on unringed branches on the same plant served as control samples.

In Expt. 2, quoted in Table 2, five branches were selected on the same plant. One branch was severed at the junction with the main stem and 2 inches of phloem tissue removed. This material was extracted and served as the initial control sample. Three branches were ringed, and total nitrogen determinations carried out on the material removed by the ringing operation. The growing apices were removed and the leaves above the rings darkened. The remaining branch was used as a final control and was left intact until the completion of the experiment when a sample of phloem was extracted for the soluble-nitrogen fractions. Four days after ringing, samples of phloem were taken from the region immediately above the rings, pooled into one sample and extracted for soluble-nitrogen compounds.

TABLE 2

Accumulation of Nitrogen Fractions in Phloem Tissue after Ringing (as mg. N per g. fresh weight)

Control samples	Total N	Sol. N	Total amide	Total amino-acid	Free amino-acid	Peptide	Sol. protein
Initial control . . .	0·92	0·42	0·11	0·15	0·04	0·13	0·04
Final control . . .	0·88	0·32	0·11	0·17	0·06	0·02	0·02
Mean of controls . .	0·90	0·37	0·11	0·16	0·05	0·08	0·03
Ringed sample . .	1·64	1·10	0·34	0·48	0·14	0·10	0·18
Increase on ringing . .	0·74	0·73	0·23	0·32	0·09	0·02	0·15

TABLE 3

Effect of Removing Growing Apices on the Accumulation of Nitrogen in the Phloem Tissue after Ringing (Estimates as mg. N/g. fresh weight)

	Initial	5 days after ringing	Increase
Branch 1. Growing points intact . . .	0·87	1·08	0·21
Branch 2. Growing points removed . . .	0·99	2·08	1·09

It can be seen that the increase in total nitrogen content on ringing could be accounted for as increases in the soluble-nitrogen fractions. This might be explained by assuming that either all the soluble-nitrogen fraction are translocated together by some mass-flow mechanism or that certain mobile forms of nitrogen are present, whose direction of movement may be governed by prevailing concentration gradients, which on accumulation above the rings are converted into the other nitrogen fractions. Such a redistribution might be expected to maintain the existing concentration gradients.

However, before discussing experiments on which possible gradients were examined, it should be noted that the accumulation of organic nitrogen in the phloem tissue after ringing does not occur, or is greatly reduced in amount if the growing apices are left intact at the time of ringing. For example, in the experiment quoted in Table 3, two stems of the same plant were ringed. On one branch, the growing apices of the stems were left intact; on the other they

were removed. The total nitrogen content was determined on samples removed by the ringing operation and on phloem tissue taken immediately above the rays, 5 days after ringing.

This suggests that the export of nitrogen from the leaves moves preferentially to the growing areas rather than to the lower stem region. Consequently, if the direction of this movement is determined by a prevailing concentration gradient it should be possible to demonstrate such gradients between leaf and apex and leaf and lower stem phloem region.

Samples of these regions were therefore taken both before and after ringing and extracted for soluble-nitrogen fractions. The results are shown in Table 4.

TABLE 4

Gradient in Nitrogen Compounds between Leaf and Stem Phloem
(*as mg.* N *per g. fresh weight*)

Initial samples	Total N	Sol. N	Protein N	Total amide	Free amino-acid	Peptide	Sol. protein
Leaf	3·88	0·40	3·48	0·15	0·20	0·05	0·03
Upper stem phloem. .	—	1·24	—	0·46	0·10	0·13	0·18
Lower stem phloem. .	0·98	0·31	0·67	0·08	0·10	0·04	0·10
Final ringed plants							
Leaf	2·28	0·36	1·92	0·14	0·08	0·0	0·0
Lower stem phloem. .	1·20	0·62	0·58	0·11	0·05	0·21	0·14
Final control plant Not ringed							
Lower stem phloem. .	1·00	0·32	0·68	0·07	0·01	0·05	0·12

Two branches were ringed and the growing points removed. Phloem samples were removed from a similar branch (initial sample). Leaves were also collected from this branch, together with two leaves from each of the ringed branches and two leaves from a further branch to be sampled at the end of the experiment (final control sample).

Five days after ringing, phloem tissue was taken from above the original rings and analysed for the various nitrogen fractions.

The results indicated that an accumulation of amino-acids and peptides occurred on ringing with smaller increases in the amide and soluble-protein fractions. There has been a loss of protein from the leaf. In the initial samples, positive gradients between leaf and lower stem existed for total nitrogen, amides, amino-acids, and peptides. Between leaf and stem apex the only positive gradient was for amino-acids. As a result of darkening the leaves, the amino-acid fraction in the leaf increased in concentration and in the experiment the gradient in free amino-acids has been maintained during the accumulation.

The extracts were examined, using two-dimensional paper chromatography with phenol-ammonia and butanol-acetic as developing solvents. Diagrams of these chromatograms are shown in Figs. 1*a* and *b* and show the increases in amino-acids occurring in the stem after ringing and indicate the presence in

the stem of a number of amino-acids after ringing which could not be detected before ringing.

These experiments suggest that amino-acids and amides play an important role in translocation; whether peptides are also translocated or whether the accumulation is due to the presence of increased amounts of amino-acids above the rings remains obscure.

FIG. 1. Tracings made from photographs taken of the chromatograms, which were developed with phenol-ammonia in the upward direction and with butanol-acetic acid-water in the other. *a*, *Pelargonium* phloem extracts, before ringing; *b*, *Pelargonium* phloem extracts, after ringing; *c*, *Vicia faba* stem extracts, before ringing; *d*, *Vicia faba* stem extracts, after ringing. Key to numbering of amino-acid traces: 1, Aspartic; 2, glutamic; 3, serine; 4, asparagine; 5, glycine; 6, threonine; 7, alanine; 8, glutamine; 9, γ-NH_2 butyric; 10, valine; 11, tyrosine; 12, leucine; 13, tryptophane; 14, arginine; 15, histidine; 16, lysine; 17, proline.

Experiments with Vicia faba *plants*

These experiments were designed to extend the results obtained in the *Pelargonium* experiments. In *Vicia faba* plants, however, xylem and phloem tissue cannot be separated for chemical analysis or can ringing be achieved by removal of a ring of extra cambial tissue. However, phloem translocation may

be interrupted by girdling a small region of the stem with a steam jet and in this way the accumulation of organic-nitrogen compounds above the killed regions of the stem may be demonstrated.

Plants of approximate uniform size and age were divided into:

1. *Initial control groups.* Three separate groups each of 6 plants were used. From each plant, 2 inches of stem immediately above the basal leaflets were removed and the material from the 6 plants of each group combined into 1 sample.

TABLE 5

The Accumulation of Organic Nitrogen Compounds
(as mg. N per g. fresh weight)

	Total N	Sol. N	Prot. N	Total amide	Free amino-acid	Soluble protein	Peptide	Nitrate
Initial controls	3·44	2·44	1·00	0·93	0·14	0·14	0·3	0·04
Final controls	3·60	2·45	1·15	0·93	0·13	0·26	0·2	0·04
Mean of controls	3·52	2·45	1·08	0·93	0·14	0·20	0·25	0·04
Ringed 1	4·75	3·74	1·01	1·40	0·33	0·41	0·20	0·04
Ringed 2	4·32	3·42	0·90	1·18	0·22	0·64	0·20	0·04
Mean of ringed samples	4·54	3·58	0·96	1·29	0·28	0·53	0·20	0·04
Increase	1·02	1·13	−0·12	0·36	0·14	0·33	—	—

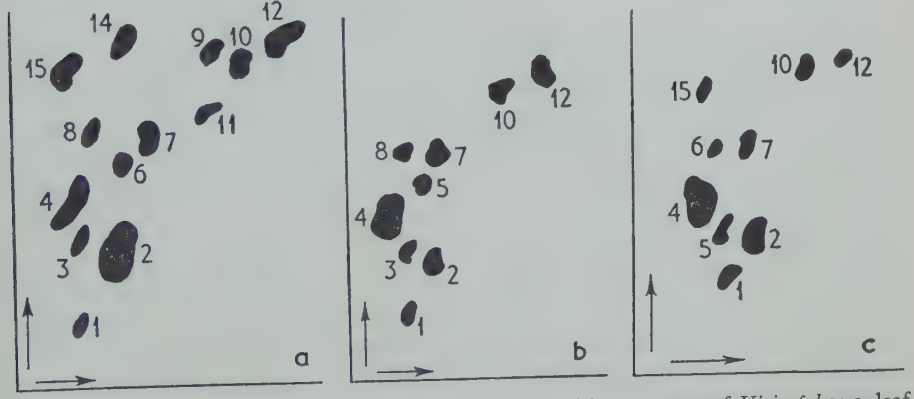

FIG. 2. Gradients in amino-acids in the leaves, upper and lower stems of *Vicia faba*: a, leaf extract; b, upper-stem extract; c, lower-stem extract. Details and numbering as in the previous figure.

2. *Ringed groups.* Twelve plants divided into 2 groups of 6 plants each were girdled with a steam jet. The growing apices of the stem were removed at the time of girdling, which was performed immediately above the basal leaflets.

3. *Final control groups.* This consisted of 1 group of 6 plants. The growing apices were removed at the commencement of the experiment; otherwise the plants were left intact until the end of the experiment.

During the experimental period the plants were kept in a greenhouse and were watered daily. All the plants remained healthy and there was no indication of leaf-wilting in the ringed groups. Three days after girdling two inches of stem were removed from immediately above the girdled region of the stem

in the ringed groups and from a corresponding region in the final control groups. The results of the analyses for the nitrogen fractions for the different samples are given in Table 5.

As a result of girdling, amide, amino-acid, and peptide fractions increased in concentration in the stem. No increase in the soluble-protein fraction occurred. Chromatograms of the amino-acid fractions of the ringed and control groups are shown in Fig. 2*a*, *b*, which confirmed the general increase in amino-acids in the experimental plants. Some of the 'spots' on the chromatograms have appeared in positions which could not be ascribed to known amino-acids and may be due to short-chain peptides.

TABLE 6

Initial Gradients at the Time of Girdling
(estimates as mg. N per g. fresh weight)

Initial	Sol. N	Total amide N	Free amino-acid N	Peptide N	Soluble Prot. N
Upper stem . . .	2·15	0·75	0·16	0·29	0·27
Lower stem . . .	1·94	0·61	0·05	0·32	0·27
3 days after ringing					
Control group . . .	1·94	0·71	0·05	0·31	0·28
Ringed group . . .	2·54	0·88	0·15	0·30	0·27

Having demonstrated the accumulation of nitrogen in the stem after girdling, the gradients in the stem and leaves of the various nitrogen fractions were determined. The results are shown in Table 6.

These results indicate that, at the time of ringing, a large positive gradient between upper and lower stem in amino-acids existed and that, after ringing, this fraction had accumulated above the girdled region. It is interesting to note that in this experiment no accumulation of peptides occurred.

Extracts of leaf, upper and lower stem tissue were chromatogramed and the results shown in Fig. 3*a*, *b*, *c*. The amount of extract applied to the chromatograms was adjusted to give equal concentrations on a per gram fresh-weight basis. The result confirms the presence of a higher concentration of amino-acids in the leaves than in the stems and the existence of a positive gradient of amino-acids between upper and lower stem regions. It is interesting to note that certain amino-acids were present in the leaves but were not detected in the stem samples.

DISCUSSION

The problem of the translocation of organic-nitrogen compounds in plants involves the identity of the actual nitrogen compounds being translocated and the mechanism involved. It might be expected that a solution of either problem may aid in the elucidation of the other.

In the two species of plants tested, an accumulation of organic nitrogen in the stem has been demonstrated after ringing and in all experiments quoted this accumulation occurred in the soluble-nitrogen fractions. The fact that

protein did not normally accumulate in the stem when transport in the phloem was interrupted is consistent with the view that movement in the phloem is restricted to crystalloid fractions.

In no experiment could the presence of free ammonia be detected in the phloem or leaf extracts, nor was there any appreciable accumulation of nitrate in the stem after ringing. However, the concentrations of total amides, amino-acids, and peptides all increased after ringing. The presence of peptides in the extracts was confirmed by the increase in concentration in the amino-acids after acid hydrolysis of the soluble-nitrogen extracts. In one experiment with *Vicia faba*, accumulation of amino-acids occurred without any increase in the peptide fraction. The possibility exists therefore that the increased concentration of the peptide fraction observed after ringing may be due to the accumulation of amino-acids above the rings and not due to the translocation of peptides as such. The rather spasmodic occurrence of the peptides in the plant extracts would perhaps favour this conclusion.

The results obtained therefore indicate the importance of the amino-acids in the transport of organic nitrogen and that the direction of movement of these compounds may be determined by prevailing concentration gradients shown to exist between the leaf and regions of the stem. However, as certain amino-acids were present in the leaves but not in the stems of *Vicia* plants, the amino-acids may be translocated at different rates.

These results have revealed little or no evidence to support the hypothesis that all the organic-nitrogen compounds in the plant are translocated together and suggests that the movement of these compounds cannot be explained on the Munch mass-flow hypothesis but are in accord with the theory put forward by Mason and Maskell (1928). This suggests that the direction of movement is determined by the prevailing concentration gradients, which in these experiments appear to be of amino-acids. But it is acknowledged that the rate of translocation is much greater than the rate attributable to diffusion and therefore even if the direction of movement is determined by concentration gradients, these gradients themselves would not be sufficient to account for the known rate of translocation. Recently, however, Spanner (1958) has suggested a possible mechanism to increase the rate of movement in the sieve-tubes.

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The Growth of Apple Fruitlets, and the Effect of Early Thinning on Fruit Development

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With fourteen Figures in the Text

Abstract

The development of fruitlets of the apple varieties Cox's Orange Pippin and Miller's Seedling was investigated with particular reference to rate and duration of cortical cell division and expansion.

The fruitlet weight increases slowly for the first few days after pollination, then exponentially at a rapid rate for about 3 weeks, and then at a declining rate until harvest.

The rate of cell division is very rapid during the exponential phase of fruitlet growth, and then the rate declines, but division continues until about 12 weeks after full bloom in Miller's Seedling and for at least 6 or 7 weeks after pollination in Cox's Orange Pippin.

The cells expand exponentially until about 7 weeks after pollination, then continue to expand at a diminishing rate until harvest.

Heavy pre-blossom thinning increased fruit size and weight, affecting the rate of both cell division and cell expansion; the differences in fruit weight showed by the 4th week and in cortical cell number and cell diameter by the 5th week after full bloom.

Introduction

THOUGH a number of environmental and cultural factors have been shown to have an effect on the mature fruit size, little is known about how or when these factors are effective during the development of the fruit. For instance, it is often stated that a given factor increases fruit size, but there is little evidence whether this has been achieved by an increase in cell number or in cell size. This point may be economically important, for the work of Martin and Lewis (1952) indicates that apples with larger cells are more susceptible to storage disorders than those with smaller cells.

The apple flower enlarges rapidly during the week before full bloom (Smith, 1950), then, at full bloom, growth slows down, or ceases for a few days (Smith, 1950; Macarthur and Wetmore, 1941). After pollination the apple fruitlet begins to expand rapidly, and continues to grow steadily until, or almost until, harvest (Tetley, 1931; Tukey and Young, 1942: and many others). This steady expansion is in contrast to the periodic development of many other fruits, including for instance stone fruits (Tukey and Young, 1939; Harrold, 1935), the black currant (Wright, 1956), and the raspberry (Hill, 1958). In these fruits there is a stage of little or no expansion between the rapid enlargement after pollination and the final swelling.

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Cell division is generally considered to be completed by 3 or 4 weeks after pollination (Tetley, 1931; Bain and Robertson, 1951), or by the end of June (Macarthur and Wetmore, 1941; Smith, 1950); Pearson and Robertson (1953) found a small increase in cell number from 50 days after flowering until the end of the season, but they thought this was possibly due to experimental errors in the method of measurement. Cell expansion begins soon after pollination in the apple fruit (Smith, 1950), and continues until late in the season.

In the pear, Toyama and Hayashi (1957) found that cell division ceased about 4 weeks after full bloom in an early variety, and after about $6\frac{1}{2}$ weeks in a late variety. On the other hand, cell division in the avocado fruit continues as long as it is on the tree, the later growth of the fruit being due to cell division rather than cell expansion (Schroeder, 1953).

There is a considerable variation in the size of mature apples on a single tree. Bain and Robertson (1951) showed that this is due to differences in cell number, only the smallest fruit having smaller cells. But the difference in fruit size between trees bearing light and heavy crops has been found to be due to cell size rather than cell number (Martin and Lewis, 1952). Seasonal differences in fruit size have been related to both cell size and cell number (Smith, 1950; Pearson and Robertson, 1953). Toyama and Hayashi (1957) found that the small size of fruit from trees defoliated in the previous autumn was due to a low cell number, while fruit from trees defoliated in the spring had smaller, rather than fewer cells.

Some apple varieties tend to be biennial bearers, producing heavy crops of small fruit one year, and light crops of larger fruit in the next. With a heavy-cropping biennial variety such as Miller's Seedling it is possible to produce very large fruit by thinning the 'on-year' crop.

Materials and Methods

The Cox's Orange Pippin blossoms were hand-pollinated and labelled, so that samples of known age could be collected at frequent intervals from pollination until harvest. On each spur the second and third flowers from the base of the cluster were used; these are the blossoms in the axils of the foliage leaves on each side of the young vegetative shoot. The flowers were pollinated at the 'balloon' stage, that is, when the flower was about to open, just before the stamens and styles were exposed. In 1957 and 1958, samples were collected from five old trees, using Beauty of Bath as the pollen parent. For each sample about the same number of fruitlets were taken from each tree. In 1959 the samples were collected from a group of 32 eight-year-old trees on the root-stock Malling VII, using Lord Lambourne as pollinator. On each sampling date one fruitlet was collected at random from each tree, to form a combined sample. As there was a poor fruit-set on these trees in 1959, it was only possible to collect hand-pollinated fruitlets for the first 3 weeks after pollination; subsequent samples were taken at random from the open pollinated fruitlets, omitting any obviously over or under average size.

The Miller's Seedling fruitlets were collected from six trees; three were

hand-thinned at the late pink bud stage of blossom, thinning to singles spaced at 12 inches, leaving the second or third blossom from the base of the cluster. The other three trees were left unthinned, and samples were selected from the same position on the cluster. All the trees were in a fully 'on-year' condition at the start of the experiment, and the unthinned trees carried a very heavy crop at harvest.

The Cox blossom was pollinated on 29 April to 1 May 1957, 11 to 13 May 1958, and 27 April 1959. In 1957 samples were collected at 1- or 2-day intervals for the first 5 weeks, at up to 4-day intervals for the next 3 weeks, then at fortnightly intervals until sampling ceased. In 1958 and 1959, samples were collected twice a week for the first 3 weeks, once a week for the next 4 weeks, then about fortnightly until harvest. Five out of the six Miller's Seedling trees were estimated to be in full bloom on 5 May 1958, and although the remaining tree (an unthinned tree) was estimated to be in full bloom at least 1 day earlier, all samples were taken at intervals from 5 May. Ten fruitlets were collected at weekly intervals from each tree.

The fresh weight of the fruitlet was determined after the removal of the pedicel, sepals, petals, stamens, and styles. The fruitlet was then fixed and preserved in formalin propionic alcohol.

In the 1957 season the cell number in the cortex was determined in two ways: by estimating the total cell number, and, using the same fruitlet, by counting the number of cells across the width of the cortex. The total cell number was counted with the aid of a haemacytometer; segments were cut from two opposite sides of the fruitlet, weighed, and macerated with a 2·5 to 10 per cent. mixture of chromic and nitric acid in equal parts, the concentration and time for maceration increasing with the age of the fruitlet. The number of cells across the cortex was counted in equatorial transverse sections; two sections were cut from each of two opposite quarters of the fruitlet, using a freezing microtome for the younger fruitlets and hand sections for the older ones, and were stained with methylene blue. In each section the cell number was counted from a sepal vascular strand and from a neighbouring petal vascular strand to the epidermis, making a total of eight counts for each fruitlet. In subsequent seasons this second method has been used for all cell-number determinations, as it has proved to be as reliable as, and very much quicker than, the haemacytometer method. A calibration curve between the two methods showed a close, but not linear, relationship between them; the slope of the curve may possibly vary from season to season, following variation in the shape of the fruit and the relative proportions of cortex and core.

From the 1957 samples, the mean cell weight of the cortex was calculated from

$$\frac{\text{total fresh weight of the cortex}}{\text{total cell number in cortex}}.$$

The mean radial cell diameter for the 1958 and 1959 samples was estimated from

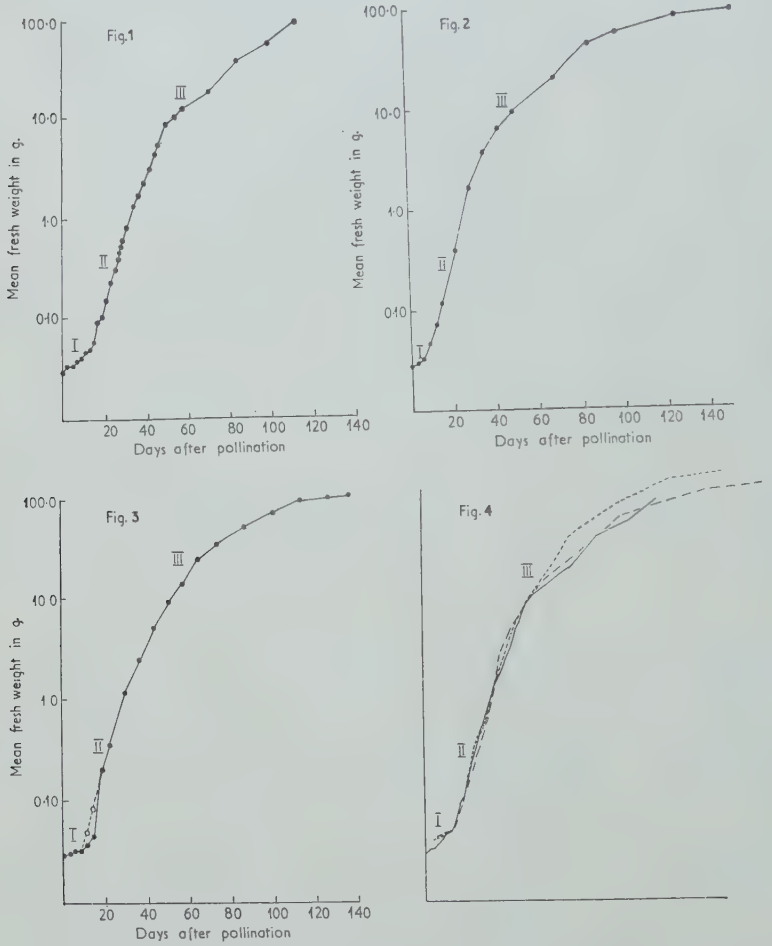
$$\frac{\text{width of cortex}}{\text{mean number of cells across cortex}},$$

the width of the cortex being the mean distance from the petal and sepal vascular strands to the epidermis.

Results

Cox's Orange Pippin

Fruitlet weight. Comparing the growth of the 1957 and 1958 Cox fruitlets, there was a relatively slow increase in fruitlet weight for the first few days



Figs. 1, 2, 3, 4. Mean fresh weight of Cox's Orange Pippin fruitlets.

Fig. 1. 1957 Crop. Fig. 2. 1958 crop.
Fig. 3. 1959 crop. -●—●- mean weight of all fruitlets, -o---o- mean weight of heavier fruitlets (for explanation see text).
Fig. 4. Growth curves superimposed at the point of the end of phase I.
—— 1957. — — — 1958. - - - - - - 1959.

after pollination (Figs. 1, 2); this lasted about 12 days in 1957, and about 6 days in 1958. Following this, there was an abrupt increase in the rate of

increase in weight, the weight increasing exponentially for about 3 weeks. The weight continued to increase until harvest, but at a declining growth-rate. For convenience in comparisons, the growth-rate may be considered to have three phases, as follows:

I. Slow increase in fruitlet weight (about 12 days after pollination in 1957, about 6 days in 1958)
II. Rapid exponential increase in fruitlet weight (about 3 weeks)
III. Declining rate of growth (until harvest)

These growth phases are not intended to imply rigid and distinct periods of growth, but merely to indicate growth trends; the distinction between phases II and III is not as clear-cut as that between phases I and II.

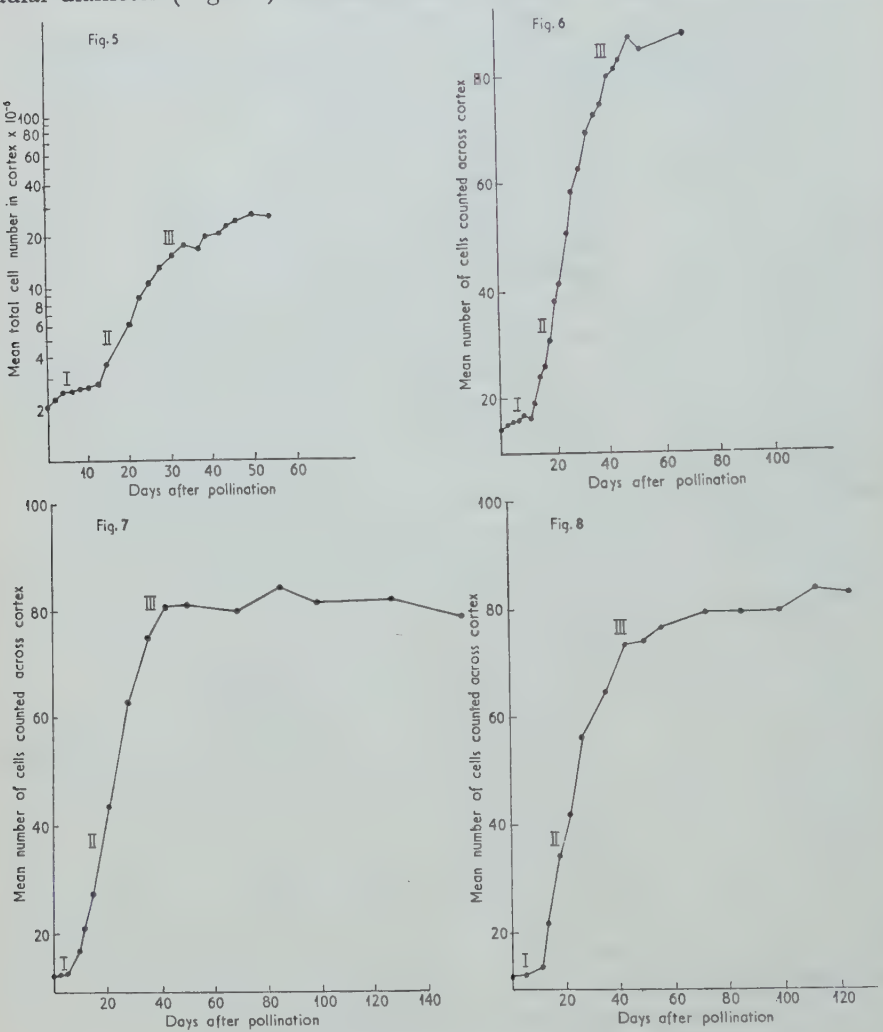
The 1959 growth curve showed a similar trend (Fig. 3), but the change in rate of growth from phase I to phase II appeared to be less abrupt. This was almost certainly due to the poor set of Cox fruitlets in 1959; there was a very heavy drop of fruitlets about 18 days after pollination, and although the fruitlets collected in the previous samples showed no signs of dropping, presumably most of them would have done so if they had been allowed to remain on the tree. Samples collected 11 and 14 days after pollination show a distinctly skew distribution of the weight about the mean, several fruitlets being considerably heavier than the majority; assuming these larger fruitlets would have remained on the tree while the majority were prospective 'drops', the mean weight of the larger fruitlets has been indicated on the graph (Fig. 3) by a broken line. The growth curve is then very similar to those of 1957 and 1958; phase I lasting about 9 days, phase II about 3 weeks.

Thus the duration of phase I varied from season to season. When the end of phase I of each of the growth curves is superimposed (Fig. 4), the rate and duration of the exponential period of growth can be seen to have been very similar in each of the three seasons; the subsequent rate of growth was similar in two seasons, but in 1959 there was a more rapid rate of growth after about 40 to 50 days after pollination.

Cell number in the cortex. Fig. 5 shows the total cell number in the cortex of the 1957 fruitlets. Cell number increased slowly from pollination until about 12 days later, that is, during phase I. For the next 3 weeks, during phase II, there was an exponential increase in cell number. Cell division continued at a diminishing rate during phase III; by the time the last sample was collected division was very slow, or possibly had ceased.

In the same fruitlets the cortical cell number was determined by the more rapid method of counting the number of cells across the cortex (Fig. 6), and this method was also used for the 1958 and 1959 fruitlets (Figs. 7, 8). In each year there was a slow increase in cell number during phase I, a very rapid increase during phase II, and a diminishing rate of cell division during the first 2 or 3 weeks of phase III. In 1958 there appeared to be little or no further change in cell number, but in 1959 there was a slow increase until at least 113 days after pollination.

Cell weight and size. Fig. 9 shows the mean cell weight in the cortex; there was a rapid exponential increase throughout phases II and III until 53 days after pollination, which was the latest sample measured that year. The mean radial diameter (Fig. 10) increased very rapidly during phase I, phase II,



FIGS. 5, 6, 7, 8. Mean number of cortical cells in Cox's Orange Pippin fruitlets.

Fig. 5. Total cell number in cortex, 1957 fruitlets.
Fig. 6. Number of cells counted across cortex, 1957 fruitlets.
Fig. 7. Number of cells counted across cortex, 1958 fruitlets.
Fig. 8. Number of cells counted across cortex, 1959 fruitlets.

and the first few weeks of phase III, that is, throughout the periods of rapid cell division. The cells continued to extend until harvest, though at a decreasing rate. After about 40 to 50 days from pollination the rate of cell expansion was more rapid in the 1959 fruitlets than in those of 1958.

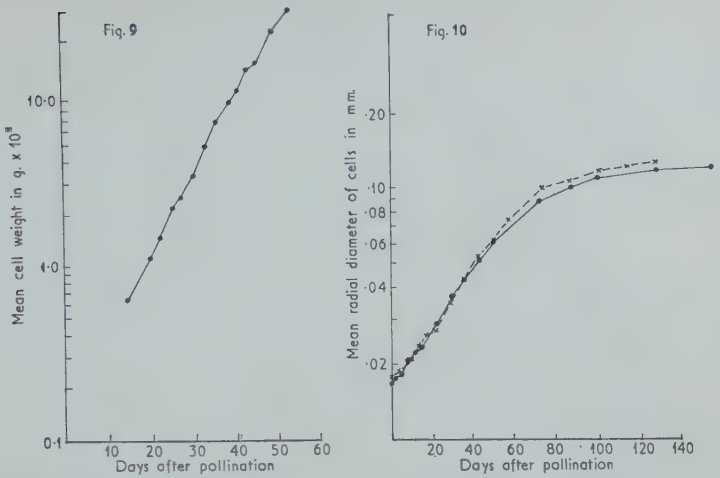


Fig. 9. Mean cell weight in cortex of Cox's Orange Pippin fruitlets in 1957.
Fig. 10. Mean radial diameter of cells in cortex of Cox's Orange Pippin fruitlets.
●——● 1958. ×— —× 1959.

Miller's Seedling

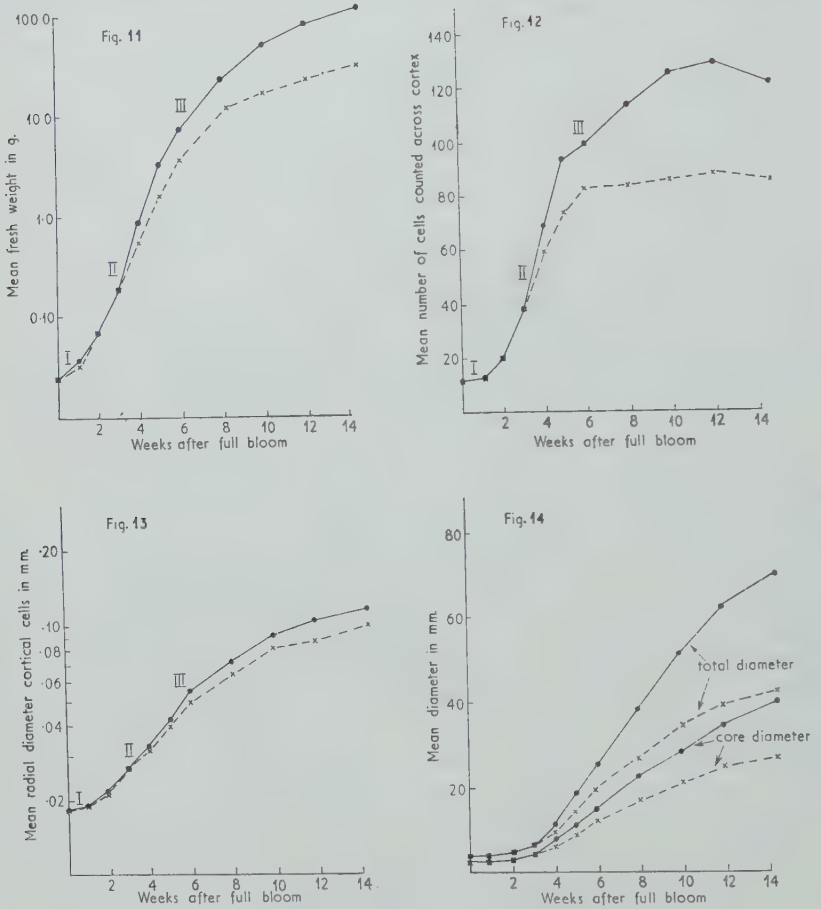
The development of the Miller's Seedling fruitlets appears to be very similar to that of the Cox's Orange Pippin (Figs. 11, 12, 13); there was a slow increase in fruitlet weight during the first 1 or 2 weeks (phase I), then a very rapid increase in growth until about 4 or 5 weeks after full bloom (phase II), followed by a decline in the rate of growth until harvest (phase III). The number of cortical cells increased relatively slowly during phase I, and very rapidly, probably exponentially, during phase II. The cells continued to expand exponentially for the first 2 or 3 weeks of phase III, while the rate of cell division declined. There was an upward trend in cell number from 6 until 12 weeks after pollination, significant at the 5 per cent. level in fruitlets from unthinned trees, and much more marked in fruitlets from thinned trees. The cell number across the cortex of fruitlets from thinned trees increased by about 30 per cent. during this time, implying that the total cell number in the cortex must have been about doubled after 6 weeks from pollination. The apparent decline in cell number from 12 weeks until harvest was possibly due to differential fruit drop, the larger fruit tending to drop first.

At harvest, the fruit from thinned trees was four times as heavy as that from unthinned trees; Fig. 14 shows that this was due to a difference in the diameter of the core as well as to the difference in cell size and cell number in the cortex.

By 5 weeks after full bloom there were significant differences in fruitlet weight,[1] cell number across cortex,[2] and cortical cell diameter[3] between fruitlets from thinned and unthinned trees. A difference in fruitlet weight was shown by 4 weeks after full bloom,[3] but the significance of differences in cell number and in cell diameter falls below the 5 per cent. level at this time.

[1] $P < 0{\cdot}001$. [2] $P < 0{\cdot}05$. [3] $P < 0{\cdot}01$.

Hence from 3 or 4 weeks after full bloom, or possibly even earlier than this, there was a faster rate of cell division and cell expansion in the fruitlets on thinned trees.



FIGS. 11, 12, 13, 14. Development of Miller's Seedling fruitlets.

●——● fruitlets from thinned trees.
×— —× fruitlets from unthinned trees.

Fig. 11. Mean fresh weight of fruitlets.
Fig. 12. Mean number of cells counted across cortex.
Fig. 13. Mean radial diameter of cells in cortex.
Fig. 14. Mean diameter of fruitlets and diameter of core.

As previously mentioned, one of the unthinned trees was in full bloom at least a day earlier than the other trees, so the fruitlets from this tree were larger than those from the other two unthinned trees on the same date. If the fruitlets had been hand-pollinated so that the samples were of a known age, then the earlier differences between treatments would probably have been more apparent.

Discussion

For the first few days after pollination there is a slow increase in fruitlet weight and in cell number in the cortex, with a relatively rapid increase in cortical cell size. The length of this phase of slow growth varies from season to season; since fertilization occurs several days after pollination in the apple, it is possible that this phase may be the time lapsing before fertilization, and its duration may depend on the rate of pollen-tube growth.

This phase of slow growth is followed by an abrupt increase in the rate of growth; the fruitlet weight increases exponentially for about 3 weeks, and then at a diminishing rate until harvest. The cortical cells divide very rapidly during the exponential phase of fruitlet growth, and although the rate of division began to slow down about 4 weeks after pollination, cell division continued in the Miller's Seedling fruitlets until 12 weeks after full bloom, that is, almost until harvest in this variety. In the Cox's Orange Pippin fruitlets cell division continued until at least 6 or 7 weeks after pollination, and probably longer than this, but a small change in the total cell number may not have been detected by the method used, as it would imply a very small change in the number of cells counted across the cortex.

The cortical cells expand exponentially for about 7 weeks after pollination, that is, throughout the phases of exponential and declining rates of cell division. Subsequently the cells continue to expand until harvest, but they do so at a diminishing rate.

An increase in both cell size and cell number was produced by severely thinning Miller's Seedling trees at the pink bud stage of blossom. At harvest, the fruits from thinned trees were about four times as heavy as those from unthinned trees, there were about three times as many cells in the cortex, and the cells were 25 per cent. wider, implying that the cell volume was about doubled.

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